

GA – 73rd Annual Meeting 2025, Naples, Italy

31 August – 03 September 2025

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73rd International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research (GA)

Date/Venue:

31.–03. September 2025, Naples

Congress President:

Alessandra Braca and Nunziatina De Tommasi

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73rd International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research (GA) Jointly with Società Italiana di Fitochimica (SIF)

Bibliography

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The 73rd International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research (GA) Jointly with Società Italiana di Fitochimica (SIF) was hosted at the Royal Continental Hotel in Naples from the 31st of August to the 3rd of September 2025 with more than 700 participants. After exactly 20 years the GA Society selected again the Italian peninsula and in particular Naples as the 2025 Annual Congress location, this year jointly with SIF.

The main scientific topics of the event covered these aspects of the natural products research:

1. Ethnobiology – Ethnobotany – Ethnopharmacology
2. Chemistry and bioactivity of natural products (including terrestrial and marine source)
3. Analysis and authentication – Quality control – Metabolomics
4. Bioengineering, bioinformatics and chemoinformatics in natural products drug discovery
5. Biodiversity, circular bioeconomy, innovative green extraction procedures and climate change
6. Herbal medicinal products, natural cosmetics, nutraceuticals, and food supplements from conception to the market – Botanical safety assessment
7. Essential oils – Clinical and environmental uses
8. Cutting edge technologies in natural product drug discovery, formulation and development
9. Fungi as medicines and functional foods – their potential and risks
10. Botanicals in veterinary medicine and feed

The meeting started on Sunday 31st of August with four Pre-Congress events:

- the Workshop: **Sustainable Botanical Ingredient Production in a World of Climate Change: Impact on Quality and the Long-Term Viability of the Herbal Trade**
 - the **Early Career Research Workshop (ECRW)**
 - the **African Research Network (ARN)**
 - the Plant for Health event **Real-world data to document the use of herbal medicinal products in children: an update**
- The scientific program of the Main Conference included:





- 14 Keynote Lectures
- 86 contributed Short Lectures
- 573 poster
- the Round Table **Revolutionizing Data Sharing Advances NP Research** on Tuesday 2nd September afternoon
- the GA2025 Science Pitch Slam on Tuesday 2nd September afternoon
- the Botanical Safety Consortium (BSC) Session on Wednesday 3rd September morning

The meeting proved to be an outstanding and memorable experience, characterized by a joyful and vibrant atmosphere. It featured inspiring talks by distinguished speakers, high-quality poster presentations, and rich opportunities for networking and meaningful exchanges with colleagues. Participants were highly multidisciplinary and represented 66 countries from all continents. Attendees ranged from senior academics to early-career researchers, as well as representatives from regulatory bodies and industry, including many non-GA and SIF members.

Overall, the meeting served as an excellent forum for scientific discussion, networking, and active engagement. The event was supported from many international and Italian sponsors and offered a great platform for presenting products, technology, services, and employment. We are grateful to them: without their significant contribution the event could not have been such a success.



We would like to thank everyone who responded to the meeting call and travelled from far and wide, starting from the distinguished speakers, with their inspiring lectures, to the students and academics who carried their posters across the world to Naples. In addition, we appreciated the contributions of the Organizing Committee, the Scientific Committee, the Local Organising Committee, and the Green Team volunteers. We are also grateful to the Organizing Secretariat Yes Meet that supported us in delivering excellence throughout planning and execution of the event. We would also like to sincerely thank the University of Naples “Federico II” for granting us access to the magnificent historic buildings of the Parthenope Congress Center.

Last, but not least, we are deeply grateful to the Executive Council and Advisory Board of the Society for Medicinal Plant and Natural Product Research, as well as to the Advisory Board of the Società Italiana di Fitochimica, for their invaluable support before and during the event. We also thank Thieme Publishers for publishing the GA2025 conference abstracts in *Planta Medica*.

On behalf of the GA2025 Organising Committee,
Prof. Alessandra Braca & Prof. Nunziatina De Tommasi

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Sustainable Botanical Ingredient Production in a World of Climate Change: Impact on Quality and the Long-Term Viability of the Herbal Trade

Sunday, August 31, 2025

SB1 Framing the issues: quality and sustainability in sourcing medicinal plants

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DOI 10.1055/s-0045-1814778

Despite what botanical ingredient suppliers and herbal dietary supplement manufacturers may claim, for the most part, price and quality are the primary drivers in sourcing herbs from around the world. Investing in sustainable and ethical sourcing, i.e., paying fair wages, considering the ecological impacts of wild harvesting, supporting regenerative farming practices that consider ecological health, and reducing carbon emissions are not a primary concern for most companies. They are instead activities to invest in if and when companies have the resources to do so.

In fact, these investments are critical to ensuring the long-term supply of high quality ingredients on which this industry depends. The Sustainable Herbs Initiative has been working with stakeholders to identify the barriers to taking action around sustainable and ethical sourcing and to initiate action to reduce and address the negative impacts from botanical ingredient production. The talk will present an overview of these barriers and the initiatives among SHI members, including – among others – jointly investing in mapping Scope 3 emissions to reduce costs for individual companies and move more quickly to decarbonization of the production; developing deeper relationships between brands and suppliers to help address quality challenges in sourcing; and developing and sharing best practices to address the quality challenges. The goal of all of these initiatives is to demonstrate that so-called sustainability is not an additional investment but is integral to the core business of herb companies seeking to produce high quality botanical products.

SB2 Medicinal plants and natural products under the challenges of climate change: Quo vadis in research and development?

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Dramatic changes to the global climate as well as the fast rise in the demand of natural resources strongly impact all aspects of medicinal and other high value plant (and fungal) resources. The supply and sourcing of wild plant populations and cultivated resources are classical examples impacting on established and traditional production systems. All these changes have accelerated enormously over the last few decades. Research and development need to integrate a more sustainability-centred strategy into their research and development strategies [1].

Based on a scoping review by our group the current state of research on how climate change affects medicinal plants, focusing on ecological shifts, traditional uses, changes in secondary metabolites, and adaptation strategies. (Takubessi et al, unpublished). The field has been slow in embracing these challenges and the majority of all studies concentrates on the major producer and consumer countries in Asia esp. China and India. There are diverse attempts to improve the footprint of research in the field, and while this is a major task, we

need to refocus what the areas of research are we need to focus on. There is a limited appreciation for the need to incorporate sustainable, long-term sourcing into the research and development strategies if new products are to be developed. Research on sustainability and climate change is not yet a focal area and an interdisciplinary, coordinated response is urgently needed to ensure sustainability and a transdisciplinary approach is needed to ascertain a response helping to ensure reducing the impact of the field on climate change, mitigation of climate changes impact and, importantly, the integration of methods from climate change research, biogeography, human ecology and other relevant fields of research.

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SB3 Medicinal plants in a complex world: how do we bridge the “knowing, but not doing gap”?

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How much more research do we really need? Shouldn't be rather focus on how to bridge the gap between academic research and real-world problem solving? We have known about medicinal plant adulteration and over-exploitation for 2000 years. At this time, awareness of geography and plant conservation was poor. So, driving a plant species to extinction and shifting to an alternative “resource-rich” frontier for a different *Ferula* species was understandable. Today, there is no excuse for ignorance. Or for “knowing but not doing”. Published, peer reviewed research is literally at our fingertips through the internet. Including climate change. Since Arrhenius (1896), showed that elevated CO₂ levels would cause warming at a global scale. Reviews on adulteration (Foster, 2011), on unsustainable use as one of the (multiple) drivers of adulteration and substitution (Cunningham et al., 2019), and of climate change on medicinal plants (Applequist et al., 2020) are all available. We no longer have the luxury of another 200 years, let alone 2000. We need to recognise international policy agreements goals such as WHO's “Health for all by 2000” or the UN CBD's Global Strategy for Plants Conservation (GSPC's) target that “by 2010, the decline of plant resources,....that support sustainable livelihoods, local food security and health care, [would be] halted” for what they are: unrealistic dreams distracting from real world implementation. What is urgently needed is to identify challenges to implementation. Identifying pivotal, coordinated roles for applied science, the commercial sector and policy makers to bridge the “knowing but not doing gap”.

SB5 Impact of climate change on medicinal plants in the Canadian boreal forests and Subarctic

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Climate change is rapidly impacting the Arctic, and the Boreal forest in Canada. Perception of changes is vivid amongst Inuit and First Nations who have voiced their concerns regarding the rapid pace of change and its impacts on medicinal plants. Some Indigenous people have mentioned that berries are now bigger and more watery or burnt in the tundra. A few Inuit shared their observations regarding medicinal roots, berries, leaves as tasting differently than some 30 years ago. Following Inuit's and Indigenous people's advice, we have sampled a number of medicinal plants along a latitudinal gradient: Labrador Tea (*Rhododendron groenlandicum*), Pitcher Plant (*Sarracenia purpurea*), Mountain Ash (*Sorbus decora*), Black Spruce (*Picea mariana*) as well as different berry producing plants (redberry, *Vaccinium vitis-idaea*; crowberry, *Empetrum nigrum*; bog

bilberry, *Vaccinium uliginosum*). We have used key metabolites and gene expressions to understand how environmental factors linked to latitude have modulated their concentrations. Bioassays have been used as well (antioxidant; adipogenesis). Berries were harvested between 2008 to 2011 from eight locales in the Canadian Arctic. At each sites, berries were compared between Open Top Chambers and controls plots. Although there's a trend of decreasing concentration over the years, results were not significant. Latitude did impact metabolite concentration with higher concentration at higher latitude until species reach their northern limit. A long term study is necessary to capture the direction of changes in metabolites. Medicinal plants in the Arctic are also impacted by shrubification, change in phenology, and pollinators. That's why some plants like it cold!

SB6 Climate change and its impact on the quality and trade of Indian medicinal plants: Case studies of ashwagandha and bacopa

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Health benefits of medicinal plants are attributed to their complex phytochemical constitution, particularly to the secondary metabolites. Climate change manifested in many forms like weather and rain patterns is a reality being experienced across the world and can have potential impact on growth and phytochemical composition of medicinal plants. In this context, we have studied two Indian medicinal plants widely used in supplements across the globe viz., ashwagandha (*Withania somnifera*) and bacopa (*Bacopa monnieri*) [1]. The two plants have a contrasting need in agroclimatic conditions for growth. Ashwagandha thrives in semi-arid to dry subtropical regions of India while bacopa is a moisture-loving medicinal herb that needs warm, humid, and wet conditions for optimum growth. Water logging on account of unseasonal rains and floods and shifts from dry land to irrigation based cultivation practices have shown reduction in content of withanolides in ashwagandha. Flooding and movement of water have made it difficult to certify lands for organic cultivation due to movement of pollutants and potential changes in soil compositions. These changes are making manufacturers of botanical extracts to innovate and adapt to maintain quality and trade.

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SB7 Exploring the connection between climate change, sustainable practices, and adulteration of botanical ingredients

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Estimates from systematic reviews on botanical ingredient adulteration suggest that approximately a quarter of the analyzed bulk ingredients and finished products may be adulterated. Sudden surges in demand, rapid price increases, or supply shortages are known to go hand in hand with an increased risk of adulteration. Climate change is one factor that impacts the availability of medicinal plants as extreme weather conditions may reduce yields, produce raw materials of lower quality, and bring new pests into the growing areas. One example is saw palmetto, which grows only in the Southeastern USA. According to saw palmetto extract manufacturers, climate change has a big impact on the supply chain, as in years when the saw palmetto harvest is low, fake saw palmetto extracts are more abundant. However, data to link adulteration directly to climate change are difficult to obtain. Similarly, data on the connection between

sustainable agricultural practices and adulteration are not readily available. An evaluation of data from five publications investigating the authenticity of 76 commercial bilberry (*Vaccinium myrtillus*), broccoli (*Brassica oleracea*) sprouts, elder (*Sambucus nigra*) berry, ginkgo (*Ginkgo biloba*) leaf, and passion-flower (*Passiflora incarnata*) herb dietary supplements and herbal medicinal products shows that 41 of 70 (59%) conventional products were adulterated, while none of the six products labeled as “certified organic”, or “made with organic” were adulterated. For essential oils, the adulteration rate of conventionally produced ingredients (n = 110) was 59.1%, and 46.7% for organic oil (n = 15). However, the low number of samples available does not allow to conclude that botanical dietary supplements, herbal medicines, or essential oils made with organic ingredients have a lower adulteration rate.

SB8 Ensuring authentic and sustainable botanical ingredients in a challenging market and changing climate

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The botanical ingredients industry operates under intense scrutiny due to recurrent adulteration practices and increasing consumer and regulatory demand for authenticity, quality, and sustainability. Economic adulteration—including substitution with inferior species, dilution with inactive materials, or fortification with non-declared compounds—undermines product efficacy and consumer trust. To ensure the integrity of botanical extracts, manufacturers must implement robust systems covering the entire supply chain. This process begins in the field, forest, or wild source, where correct species and plant part identification, as well as appropriate harvesting conditions, are essential. These steps must align with good agricultural and sustainability practices to protect both product quality and natural resources [1]. Post-harvest procedures—including drying, milling, storage, and transport—must preserve the phytochemical profile while preventing contamination. A multilayered quality control system is vital. It includes classical methods (HPTLC, HPLC, microscopy) and advanced techniques like DNA barcoding and spectroscopy. These tools support phytochemical fingerprinting, identity verification, and detection of adulterants. The case of *Serenoa repens* (saw palmetto) exemplifies how organoleptic deviations, fatty acid profiles, and spectral analysis can reveal fraud such as substitution or oil adulteration [2].

Climate change further complicates the landscape, affecting harvest periods, raw material quality, and availability. Therefore, manufacturers must adopt adaptive sourcing and processing strategies, while maintaining full traceability and analytical rigor. In a competitive and evolving market, the production of high-quality, authentic, and sustainable botanical ingredients requires deep knowledge of raw materials, controlled processes, and a firm commitment to transparency and scientific validation.

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SB9 Shaping the future: integrating sustainability and policy in research and innovation

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The use of natural products is rising across sectors, driven by economic, environmental, health and societal needs. While EU sustainability directives set ambitious plans, bioeconomy strategies are expanding globally, promoting biobased solutions with focus on sustainability and circularity across value chains [1]. Emphasis is given to collaboration and awareness to accelerate innovation and advance viable new solutions through biotech and biorefinery scale-up.

Boglands are a unique habitat. They play a key role within climate change as peatlands store 1/3 of the world's soil carbon and provide water storage [2]. To promote healthier planet and population and supporting the Irish bioeconomy strategy and policy [3, 4], we unlocked the potential of Ireland's boglands as a natural pharmacy, disrupting the *status quo* and shifting their perception from a traditional fuel source to a valuable reservoir of bioactive compounds for health and wellness. Our research involved studying bogland plants, fostering collaboration across government, academia, industry and communities, while enhancing awareness of the biodiversity these landscapes offer.

As climate change continues to pose global challenges, laboratories are recognised as environmentally intensive facilities. Promoting sustainable laboratory practices is a goal endorsed by the United Nations and recently by EU funding bodies. As Irish early adopters of international green certifications, we remain committed to implementing greener practices that inspire behavioural change and reduce environmental impact, while exploring innovative research. We engage with a national network to foster a culture of sustainability by design, sharing education and best practices to drive a paradigm shift in addressing global needs.

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SB10 Regenerative agriculture: Influence on soil ecosystems and plant phytochemical composition

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Climate change has increasingly impacted soil health due to rising air temperatures and unpredictable rainfall patterns, leading to water scarcity in some areas and flooding in others. These extreme conditions disrupt the ability of soil ecosystems to function effectively. The negative effects are often more pronounced in intensively managed soils that lack robust physical structure and biological diversity. Regenerative organic agriculture offers a promising solution to these challenges. By implementing key practices such as cover cropping, the use of organic fertilizers, and crop rotations, this approach can significantly improve soil health. One of its major benefits is the enhancement of soil organic carbon content, which is closely linked to improved water retention, stronger soil structure, and increased biodiversity.

Our global review study [1] and our experiments in EROC demonstrate that regenerative organic agriculture positively influences a wide range of soil ecosystem services. These include the enrichment of plant nutrients and the production of secondary metabolites, which hold potential value for industries such as cosmetics.

In my discussion, the ways by which regenerative organic farming not only restore soil health but also strengthen the capacity of soils to deliver essential ecosystem services are highlighted — services that are crucial for the sustainability and resilience of human societies.

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Early Career Researcher Workshop

Sunday, August 31, 2025

IL1 Curiosity, passion, and the journey to a career in pharmacognosy

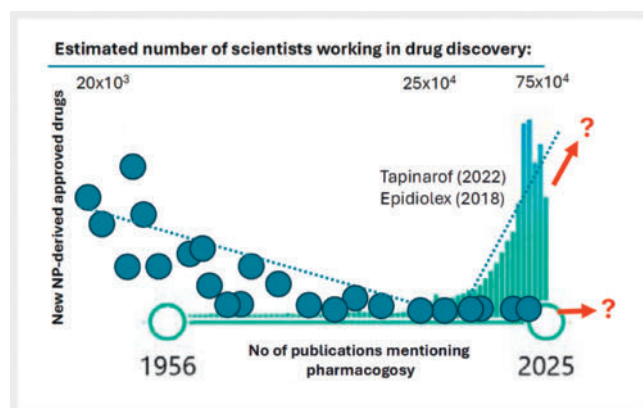
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Pharmacognosy is the branch of pharmaceutical science focused on bioactive natural products (NPs), some of which serve as drugs or drug leads. It encompasses the identification, extraction, characterization, and biological evaluation of compounds from plants, animals, and microorganisms. In this impulse lecture, I will share my passion for bioprospecting, bioactive molecules, and ethnopharmacology, which began in 1997. I will reflect on my nonlinear career path in pharmacognosy, emphasizing the value of interdisciplinary, curiosity-driven research. From the rainforests of Venezuela [1] to the politics of bioprospecting and the excitement of lab work, the journey has involved molecules, people, diseases, and emerging drug targets. I will describe my early, often naïve, experiences in drug discovery, particularly in neuropharmacology and the endocannabinoid system [2, 3]. This includes insights into translational research and the importance of start-ups and spin-offs in advancing drug development [4]. Our current research on medical cannabis and modulation of the endocannabinoid system exemplifies this approach.

Despite growing research on NPs, successful NP-derived drug development remains rare, highlighting a translational gap in pharmacognosy (► Fig. 1). I will conclude with our lab's ongoing work on Chagas disease (CD), caused by *Trypanosoma cruzi*, which affects over six million people globally [5]. Developing drugs for the chronic stage of CD is highly challenging, yet pharmacognosy may offer solutions. Natural products have shown potent antiparasitic effects, and antichagasic NPs or plant extracts could help bridge the gap between basic research and viable therapies.



► Fig. 1 Despite the increasing number of scientists worldwide mentioning pharmacognosy in their papers the actual translation of natural product (NP) drug discovery is declining steadily

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EC1 *In vitro* anti-inflammatory potential of hydrosols and essential oils from fresh female cones of five *Juniperus* spp. collected in Greece

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Needle-leaved junipers from the *Juniper* genus (Cupressaceae), are coniferous trees and shrubs with red or blue fleshy cones, distributed across Asia, Macaronesia, and the Mediterranean Basin [1]. The genus includes approximately 75 species which are widely used in the food and cosmetics industries [2]. Recognizing its medicinal properties, the European Medicines Agency (EMA) has published two monographs on *Juniperus communis* dried cones and essential oil, highlighting their traditional use as a diuretic and for digestive, gastrointestinal, and musculoskeletal relief [3, 4]. The essential oils of *Juniperus* species are rich in monoterpenes, with the bicyclic monoterpene α -pinene being the major constituent, followed by sesquiterpenes. While most research focuses on the antimicrobial activity of essential oils, studies on their anti-inflammatory activity are scarce, presenting an interesting challenge. Moreover, there is no information on the activity of the hydrosols yet. Many endemic species can be found in Greece, which can represent a source of essential oils and hydrosols for the treatment of some inflammatory disease by dermal application. So, this study investigates the fresh female cones of five Greek *Juniperus* species: *J. drupacea*, *J. communis* subsp. *hemisphaerica*, *J. oxycedrus* subsp. *deltoides*, *J. macrocarpa* and *J. turbinata*, through distillation and chemotype analysis via GC-MS. This oral presentation details the hydrodistillation protocol used to obtain both hydrosols and essential oils and, discusses the *in vitro* results related to their anti-inflammatory activities. These will be assessed in macrophages using NADPH oxidase inhibition and cell viability assays to ensure their effectiveness in reducing inflammation while confirming non-toxicity.

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EC2 Rationalizing the dosage of phytopharmaceuticals for children by extrapolating adult pharmacokinetic data using PBPK modeling: St. John's Wort as example

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Herbal medicinal products (HMPs) are effective and safe therapeutic options for children. However, many HMPs approved for the treatment of adults are under-researched in pediatric populations. Therefore, off-label use is widespread [1]. This study aimed to develop and evaluate a physiologically-based pharmacokinetic (PBPK) model for adults (PK-Sim®, OSP Suite) with hypericin from *Hypericum perforatum* as a model compound using published pharmacokinetic data from different extracts [2, 3]. The PBPK model has the potential to close knowledge gaps regarding pharmacokinetic properties of the compound and further the St. John's Wort extracts. It allows prediction of drug exposure in pediatrics based on age-related physiological differences by extrapolation of adult data. To establish a valid PBPK model, the following key-parameters were defined: (I) drug-specific properties (lipophilicity, solubility), (II) drug-biological properties (fraction unbound, binding partners, clearance) and (III) formulation-dependent factors (dissolution, lag time). The general PBPK modeling workflow was applied as described by Kuepfer et al. (2016) [2]. Various simulations were performed using different methods for partition coefficient and cellular permeability calculations. Interestingly, the two different extracts led to different PBPK models. The developed adult PBPK models successfully predicted hypericin pharmacokinetics and was extrapolated to pediatric populations following the ICH E11A guideline on pediatric extrapolation [3].

By predicting age-dependent drug exposure based on physiological differences, the model provides detailed insights into pharmacokinetic variability in adults and children. This approach bridges knowledge gaps, reduces the need for pediatric clinical trials, saves costs, optimizes drug development, and improves availability of evidence-based HMPs for pediatric use [4, 5].

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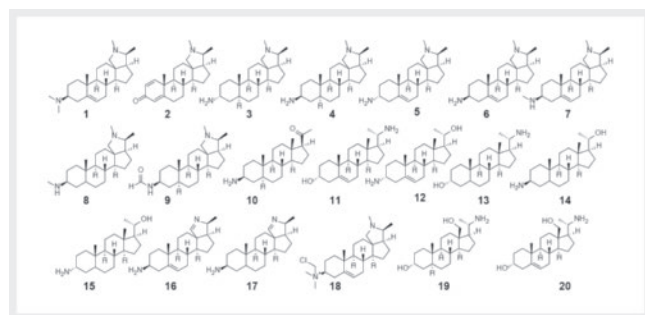
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EC3 Antiprotozoal aminosteroid alkaloids from *Holarrhena pubescens* Wall. ex G. Don

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Human African Trypanosomiasis (HAT) and Malaria are prevalent protozoan infections in tropical regions, necessitating development of new antiprotozoal drugs. In our ongoing search for new antiprotozoal aminosteroid alkaloids from the Apocynaceae family [1], we investigated the methanolic stem bark extract of *Holarrhena pubescens*. It is a tropical tree that some Kenyan coastal communities have historically used to treat various diseases including fever and stomach discomfort [2]. The crude extract, alkaloid-enriched fraction, and 15 subfractions acquired through centrifugal partition chromatography (CPC) were assessed for *in vitro* activity against *Trypanosoma brucei rhodesiense* (*Tbr*; causing HAT) and *Plasmodium falciparum* (*Pf*; causing Malaria) using standard assays [3]. Compared to the crude extract (IC_{50} = 0.89 μ g/mL, SI = 68), the alkaloid-enriched fraction displayed higher activity against *Tbr* (IC_{50} = 0.24 μ g/mL, SI = 62). Similarly, the alkaloid fraction exhibited almost 6-fold antiparasmodial activity (IC_{50} = 0.70 μ g/mL, SI = 21) compared to the crude extract (IC_{50} = 4.04 μ g/mL, SI = 15). Most of the CPC subfractions demonstrated strong activity (IC_{50} < 1 μ g/mL) against both parasites. Using prep-HPLC, UHPLC/ESI-QqTOF-MS/MS and NMR spectroscopy, 20 compounds have so far been isolated and characterized (► Fig. 1). Compounds 1–18 have been tested for their *in vitro* antiprotozoal properties against the two pathogens. Compound 1 (IC_{50} = 0.21 μ M, SI = 118) demonstrated the highest antitrypanosomal activity, while compound 9 had the strongest antiparasmodial activity (IC_{50} = 0.66 μ M, SI = 45). Isolation and antiprotozoal activity studies of more compounds are underway. The authors declare that they have no conflict of interest.



► Fig. 1 Structures of the compounds isolated from *Holarrhena pubescens*

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EC4 *In vivo* and *in silico* analysis of *Xanthii fructus* against atopic dermatitis

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Xanthii fructus (XF) is the fruit of *Xanthium strumarium* L., has been used as a traditional medicine for its bioactive properties in China, America, and Europe. In our previous study, Korean (XFK) and Chinese (XFC) XF extracts demonstrated antioxidant and anti-inflammatory effects in cell-based models, including the collagen promotion and hyaluronan synthesis. The aim of this study was to evaluate the anti-atopic effects of XFK and XFC *in vivo* and to compare these result with *in silico* analysis in order to identify potential signaling pathways [1–3]. In a DNCB-induced mouse model of atopic dermatitis, both XFK and XFC significantly reduced scratching frequency and the skin thickness of epidermis and dermis. XFK specifically led to reduction reduced in SCORAD index, transepidermal water loss and serum IgE levels, whereas neither extract significantly affected serum histamine levels. Furthermore, XFK decreased the mRNA expression of TNF- α and

IL-6, whereas XFC modulated p-p38/p38 ratio and iNOS expression. *In silico* network analysis identified oleic acid, linoleic acid, and arachidonic acid; as a key compounds linking XF with inflammation-related genes such as IL-1 β , IL-4, IL-6 and CASP3.

In conclusion, both XFK and XFC ameliorated immune response associated with atopic dermatitis via different mechanisms. These differences are likely due to geographical variation in bioactive composition. Despite these differences, both XF extracts show promising potential application in cosmetic and pharmaceutical products targeting inflammation and atopic skin conditions.

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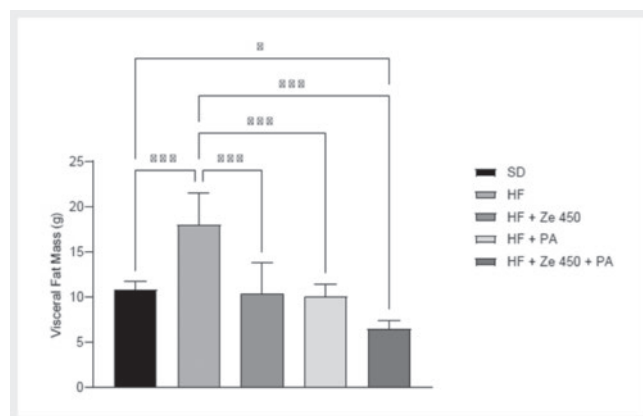
EC5 *Cimicifuga racemosa* Extract Ze 450 Counteracts High-Fat Diet-Induced Fat Gain and Metabolic Dysregulation in Female Rats

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Physical inactivity and a high-fat diet (HF) contribute to obesity and metabolic disorders, while physical activity and phytochemicals may support metabolic health [1, 2]. Ze 450, an ethanolic dry extract of *Cimicifuga racemosa* (L.) Nutt., has been shown to influence energy metabolism [3]. This study investigated how Ze 450 (130 mg/kg body weight), physical activity and combinations mitigate HF-induced metabolic alterations over 12 weeks.

Thirty female Wistar rats (n = 30) were assigned to Standard Diet (SD), High-Fat Diet (HF), HF + Ze 450, HF + physical activity (PA), or HF + Ze 450 + PA (n = 6/group). Body weight was recorded daily, and visceral fat mass and serum metabolic markers were assessed post-mortem. Rats in the PA groups had nightly access to running wheels (► Fig. 1).



► Fig. 1 Visceral fat mass (g) in different dietary and intervention groups. Data are presented as mean $\pm$ SD (n = 6 per group). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. Visceral fat mass was significantly higher in the HF group compared to the SD group (p < 0.001). All intervention groups (HF + Ze 450, HF + PA, and HF + Ze 450 + PA) had significantly lower visceral fat mass compared to the HF group (p < 0.001). Additionally, the HF + Ze 450 + PA group showed a significantly lower visceral fat mass compared to the SD group (p < 0.05).

Visceral fat and serum leptin, indicative of total body fat, were significantly lower in all intervention groups compared to the HF group, with the combination of Ze 450 and PA showing additive effects. Adipocyte size was significantly reduced in all intervention groups compared to HF and even SD. Body weight was significantly lower in the HF + Ze 450 + PA group compared to the HF group, with no other significant differences between groups. Finally, Ze 450 reduced energy intake and increased wheel running activity.

These findings suggest that Ze 450 modulates fat metabolism and improves metabolic health, with its effects further amplified when combined with physical activity.

Funded by Zeller & Söhne AG, Switzerland. The authors declare no additional conflicts of interest.

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EC7 Integration of Metabolomics in Pharmacokinetic studies: the example of *Cistus creticus*

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Natural products remain a cornerstone of drug discovery, yet they often fail to progress into pharmaceutical drugs due to the lack of comprehensive evaluation in preclinical studies [1] promising preclinical NP research is not consistently translating into actionable clinical trial (CT). *Cistus creticus* (Cistaceae) a medicinal plant known for its anti-inflammatory and antioxidant properties, among others, is commercially available as a dietary supplement [2] infectious diseases are becoming harder to treat. Hence, there is an increasing awareness of active phytochemicals with therapeutic functions. The tremendous research interest on the *Cistus* L. genus includes numerous plants used in traditional medicine by people living around the Mediterranean Sea, also resulted in some interesting discoveries and written literature. This review aimed at gathering scientific literature about *Cistus* species, describing phytochemical profiles and the various pharmacological activities. We also extensively reviewed the antimicrobial activities, including antiviral, antiparasitic, antifungal, and antibacterial potentials of Essential Oils (EO). To overcome that barrier, we conducted a pharmacokinetic study utilizing an *in vivo* mouse model to assess the absorption, distribution, metabolism, and excretion (ADME) profile of the bioactive compounds present in a standardized extract of *Cistus creticus* from Lesvos, Greece. A tailored UPLC-HRMS workflow was employed to track the extract's major constituents in mouse plasma and their metabolites as a following step. To enhance compound identification, an in-house spectral library was developed using the NIST platform, incorporating spectra from metabolites previously isolated from biological samples in our laboratory and identified through LC-HRMS and NMR techniques. Spectral library searching is currently the most common approach in terms of time efficiency and precision regarding annotation [3]. Given the lack of MS/MS spectral data for natural product metabolites, this is a first step towards their enrichment. The findings of this study not only provide insights into the pharmacokinetic behavior of *Cistus*-derived compounds but also highlight their potential as therapeutic agents. This work paves the way for further development of natural products as viable drug candidates.

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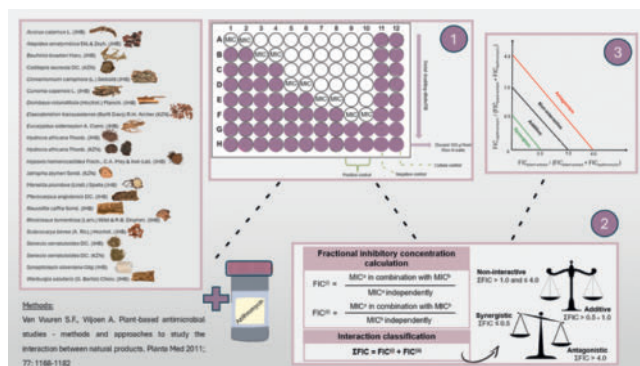
EC8 South African traditional medicinal plants and their synergistic interactions with azithromycin

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Traditional medicinal plants are frequently purchased from traditional *muthi* markets in South Africa. The Faraday market in Johannesburg (JHB), Gauteng and the Warwick market in Durban, KwaZulu-Natal (KZN), are two of the largest *muthi* markets in South Africa, responsible for traditional medicinal plant trading. Our previous studies on medicinal plants from these two markets showed some interesting interactive antimicrobial profiles with conventional antibiotics. Here we explore 22 of the ³⁴ plants investigated, which were found to be antimicrobially synergistic against *Staphylococcus aureus* (ATCC 25923) when combined with azithromycin (► Fig. 1). The minimum inhibitory concentrations were determined for the medicinal plants and azithromycin and the fractional inhibitory concentration index (ΣFIC) calculated for the combinations. The highest synergy was found for azithromycin: *Hypoxis hemerocallidea* Fisch., C.A.Mey. & Avé-Lall. against *S. aureus* (ΣFIC 0.06). Synergistic interactions were calculated at varied concentrations for a dose-response analysis, against *S. aureus* (ATCC ²⁵⁹²³). Further, the ΣFIC calculations were used for interactive profiling of nine varied combination ratios (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9) and isobolograms constructed. The brine shrimp lethality assay (BSLA) allowed for further *in vitro* toxicity analysis of synergistic ratios. Findings confirmed that synergism was dose-dependent, with ratios exhibiting varied antimicrobial profiles, and potential for toxicity. This research highlights the need for further dose-dependent investigations, inclusive of biofilm activity, mechanism of action studies and *in vivo* antimicrobial and toxicity analysis, using a varied dose-response approach.



► Fig. 1 Overview of the plant sample: azithromycin combinations and applied methods for investigating interactive antimicrobial and toxicity profiles of varied ratio concentrations.

EC9 QUALOME: A workflow integrating genotyped reference materials and advanced metabolomics as a new benchmark for quality assessment of herbal medicinal products

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The advancement of metabolomics techniques has enabled detailed characterisation of the metabolome of plant species [1]. Given their complexity, understanding the full phytochemical composition of plant matrices is crucial, especially in the case of herbal medicinal products and dietary supplements, where rigorous quality control approaches are essential to prevent adulteration and ensure their safety, efficacy, and authenticity [2] but also required to meet rising demands in safety and toxicological assessment, particularly through *in silico* approaches carried out by the industry [3]. The QUALOME initiative introduces an innovative workflow that integrates genotyped reference materials with advanced qualitative and quantitative metabolomic profiling, offering a benchmark dataset for quality assessment.

Our approach leverages high-resolution mass spectrometry (HRMS) and nuclear magnetic resonance (NMR) [4] to characterise comprehensively major and minor compounds. Semi-quantitative data is obtained through charged aerosol detection (CAD) and efficiently linked to HRMS data [5]. We showcase our workflow on herbal medicinal plants of pharmacoepoeial interest, utilizing exhaustive extraction processes across a wide polarity range.

QUALOME has been developed to qualitatively and quantitatively estimate the composition of both primary and secondary metabolites, and to provide a reference dataset that serves as a benchmark for the quality assessment of herbal medicinal products. In this study, we applied the QUALOME workflow to a selected set of medicinal plants, namely *Arnica montana*, *Echinacea angustifolia*, *Actaea racemosa* and *Eschscholzia californica*. We demonstrated its effectiveness in in-depth qualitative and semi-quantitative metabolomic characterisation.

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EC10 Identification of Kellerin as a Bioactive Principle of *Ferula assa-foetida* with Potential Anti-Angiogenic and Anti-Inflammatory Effects

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Ferula assa-foetida L. (Apiaceae) is a plant native to southern Iran and Afghanistan. Its dried resin, called asafoetida, has been used for millennia as a spice and in traditional medicine for various indications [1]. In particular, its anti-inflammatory effects have gained increasing interest in research. However, the mechanisms of action and molecular targets of these constituents are yet to be fully elucidated.

This study investigates the potential anti-angiogenic and anti-inflammatory activities of *F. assa-foetida* resin and its bioactive constituents. A methanolic extract of the resin inhibited both the proliferation of primary endothelial cells as well as the adhesion of leukocytes to the endothelial cells upon pro-inflammatory activation. Bioassay-guided fractionation identified the dichloromethane fraction as the most potent one, reducing endothelial cell proliferation with an IC₅₀ of 15 µg/mL. This fraction was further purified, which led to the isolation and structural characterization of three sesquiterpene coumarins: farnesiferol B, farnesiferol C, and kellerin [2]. Among them, kellerin showed the highest antiproliferative activity with an IC₅₀ of 6 µM; and significantly reduced leukocyte adhesion at a concentration of 5 µM. Transcriptomic analyses in primary monocytes revealed a downregulation of inflammation-related genes after kellerin treatment. Further computational analysis using decoupleR [3] predicted the involvement of transcription factors associated with inflammation, including STAT3, FOS and JUN.

Ongoing studies, including proteomic analyses, aim to elucidate the underlying molecular mechanisms and identify involved target proteins. Taken together, our work highlights kellerin as a promising bioactive lead compound with dual anti-angiogenic and anti-inflammatory potential warranting further pharmacological research.

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EC11 Integrating utargeted metabolomics with biochemometrics to uncover antibacterial naphthyl derivatives in *Guiera senegalensis* leaves

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Multidrug resistant bacteria (MDR) are one of the worldwide emerging threats to Public Health. *S. epidermidis* is a MDR pathogen and a major contributor to hospital-acquired infections, particularly due to its ability to form biofilms. Clinically, MDR *S. epidermidis* complicates treatment of device-associated infections and sepsis, requiring advanced diagnostics and therapies to mitigate its impact in healthcare settings [1].

Plant-derived natural products, due to their inherent chemical diversity, offer a promising avenue to address MDR [2]. Modern technologies are essential to revitalize ethnopharmacological tradition by accelerating the discovery pipeline [3]. In this study, we present the identification of anti-bacterial naphthyl derivatives from *Guiera senegalensis* leaves, used in different African countries to treat infectious and chronic diseases. Our combined approach of untargeted LC-HRMS and Molecular Networking-based metabolomics and NMR-based biochemometric analysis allowed both the comprehensive snapshot of the metabolome of the extract and the detection of key NMR-features responsible for the antibacterial activity exerted by the extract. NMR-fingerprint driven the chromatographic purification allowing the isolation of 7 naphthyl derivatives, including the new naphthyl-butenone guieranone C, and two unprecedented naphtho- γ -pirones, namely guierapyrone A and B. Isolated metabolites were tested for their anti-bacterial activity against *S. epidermidis* and oxacillin resistant *S. epidermidis* unveiling guieranone A as a good candidate for fighting staphylococcal infections.

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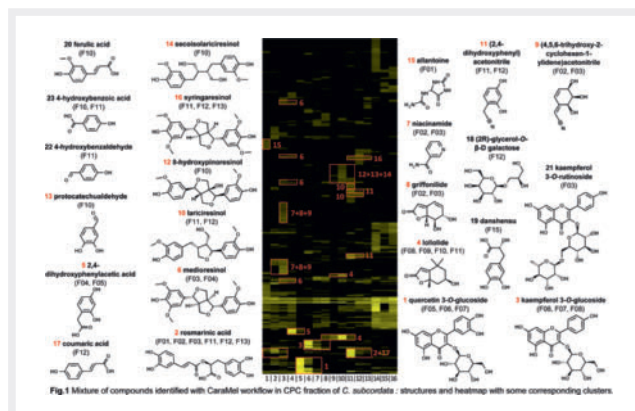
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EC12 Chemical profiling of complex mixtures from Polynesian plants for mapping wound-healing properties

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Polynesian plants such as *Calophyllum inophyllum*, *Gardenia taitensis*, and *Cordia subcordata* are widely used in traditional medicine, especially for skin care applications (wounds, burns, pimples, dermatoses) [1]. Inspired by those uses, this study aims to assess their wound-healing properties on skin cells and to quickly characterise metabolites in mixtures for a cosmetic and pharmaceutical valorisation. Therefore, plant extracts were obtained using ultrasound-assisted extraction with ethanol: water (7:3, v/v) solvent [2]. This eco-extraction method afforded efficient yields and is suitable for cosmetic valorisation. Plant crude extracts were first submitted to liquid-liquid partition using stepwise gradients, and the resulting EtOAc extracts were fractionated by centrifugal partition chromatography (CPC). Then, scratch wound healing assays on keratinocytes (HaCaT) and fibroblasts (CI-huFIB) were performed on extracts and fractions at micromolar concentrations. Promising results were obtained by enhancement of migration and proliferation of skin cells at non-cytotoxic concentrations for these three plant extracts. Dereplicative methods were performed to profile the chemical composition using molecular networking within LC-MS² analysis

combined with CaraMed workflow through ¹³C NMR analysis [3]. ¹³C signals of CPC fractions were used to create heatmaps using hierarchical clustering analysis with resulting clusters corresponding to molecular structures (► Fig. 1). This method allowed accelerated characterisation, avoiding purification processes and leading to annotations and molecular identifications with high confidence. Finally, the correlation between wound-healing properties and chemical composition was performed for mapping biological activities. These results highlight the potential of Polynesian plants from cosmetopoeia and pharmacopoeia as a source of bioactive compounds for skin care applications.



► **Fig. 1** Mixture of compounds identified with CaraMed workflow in CPC fraction of *C. subcordata*: structures and heatmap with some corresponding clusters.

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African Research Network Workshop on Natural Products Research

Sunday, August 31, 2025

IL2 The commercialization and industrialization of herbal medicine in Africa

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Despite the fact that more than 80 % of Africans rely on herbal medicine for their health and welfare the commercialization and industrialization of the African herbal medicine sector has been very slow compared with the rest of the world. Further despite Africa having a vast medicinal plant and cultural diversity, less than 5 % of the 45,000 plant species found on the continent have been developed for formal markets at a national or international level [1]. This oral presentation will examine the constraints and the opportunities for African

herbal medicine focusing on the prime importance of developing quality control standards. It will focus on the work of the Association of African Medicinal Plants Standards (www.aamps.org) which published the first pan-African Herbal Pharmacopeia (AfHP) in 2010. The latest edition of this book is being published in November 2025. The AfHP aims to enhance the credibility and global competitiveness of African herbal products by providing standardized guidelines for research, manufacturing, and regulation [2]. The presentation will include case studies of African herbals that have been successfully marketed worldwide and examine the reason for their success and lessons to be learnt for those entering this industry.

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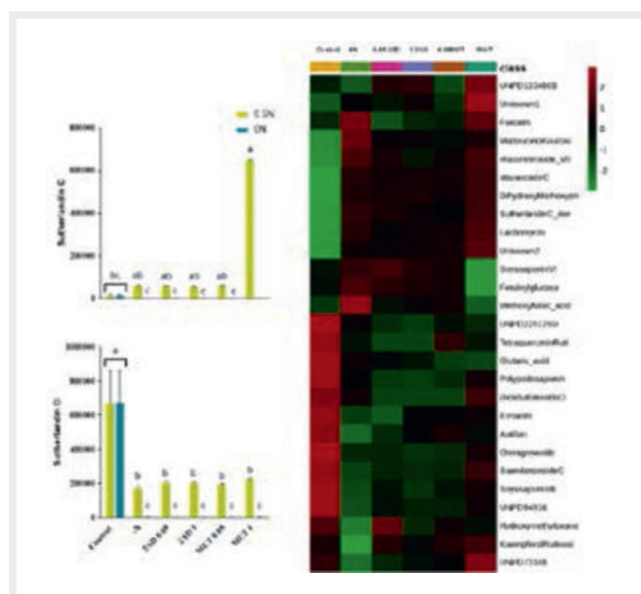
AR1 Metabolite changes in *Sutherlandia frutescens* (L.) in response to nutrient stress and cytokinin oxidase/dehydrogenase inhibitor application

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Cytokinin functions either at molecular, physiological, or morphological level, and are linked to a variety of plant responses related to abiotic stresses [1]. Cytokinin oxidase/dehydrogenase (CKX) inhibitors are known to promote growth, nutrient signalling and yield in stressed and non-stressed model plants. Besides the beneficial role of CKX inhibitors during plant growth and development, they have also been shown to be safe and can be applied in agriculture [2, 3]. Hence, the aim of this study was to investigate the role of CKX inhibitors in mitigating the negative effect of nitrogen deficiency stress in *S. frutescens*, a medicinal plant legume. Nitrogen stress was triggered by halving ($\frac{1}{2}$) or completely eliminating ($-N$) essential nutrients (NH_4NO_3 , KNO_3) in *in vitro* propagation medium. Nitrogen deficiency ($-N$) significantly reduced shoot production in plantlets. Whereas CKX inhibitors (0.05 and 1 nM) counteracted the effect of nitrogen deficiency by eliciting a two-fold increase in plant leaf number and shoot height. Using liquid chromatography-mass spectrometry analysis (targeted and non-targeted metabolites), plantlets showed a significant decrease in biomarker compounds such as sutherlandins (C, D and derivatives), ermanin and soyasaponins, especially in treatments deficient of nitrogen (► Fig. 1). Amongst the tested compounds, CKX inhibitors especially 1 nM MUT upregulated a number of secondary metabolites in plants that were exposed to nitrogen deficiency. The current findings demonstrate that CKX inhibitors have a potential to mitigate the impact of nutrient stress especially during plant growth and development by triggering the upregulation of primary and secondary metabolites.

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► Fig. 1 Metabolite changes in *Sutherlandia frutescens* plantlets exposed to nutrients stress after the application of CKX inhibitors.

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AR2 Molecular networking -guided isolation of compounds from *Keetia venosa* (Oliv.) Hiern leaves and their antibacterial activities

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The global incidence of antibacterial resistance represents a significant public health challenge, with an estimated number of 4.95 million deaths in 2019, predominantly in developing countries [1]. This emphasises the urgent necessity to identify novel molecules with antibacterial properties to address this concern. Medicinal plants have been identified as playing a crucial role due to their rich chemical diversity and bioactivity [2]. Nonetheless, the conventional approach to natural product isolation remains challenging, as evidenced by the technical limitations associated with in-depth screening of biomaterials. In this study, we have employed a molecular networking -guided approach aiming at the in-depth chemical profiling and isolation of new potential antibacterial compounds from a medicinal plant *Keetia venosa* leaves. These plants are used in traditional medicine to treat bacterial and parasitic infections.

This applied method led to the isolation and characterization of 18 compounds, including 4 previously undescribed. The structures of these compounds were elucidated through a comprehensive analysis of their physical, HR-MS/MS and NMR spectroscopic data. Several additional compounds were putatively identified by comparing their MS2 features with analogous structures in established databases. Moreover, the antibacterial activity of extracts, fractions and isolated compounds were evaluated against a range of Gram-positive and -negative bacteria. Some fractions and isolated compounds displayed promising antibacterial activity against Gram-negative bacteria such as *K. pneumoniae*, *S. enterica* and *P. aeruginosa* with MIC values ranging from 3.9–62.5 µg/mL.

The molecular networking-guided applied methodology speeds up the dereplication, targeting and isolation of natural products from plant extracts.

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AR2 bis *In silico* bioprospection of phenolics against penicillin binding protein (PBP1A) of *Acinetobacter baumannii* for interventional therapy

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Infections caused by carbapenem-resistant *Acinetobacter baumannii* can result in death within 48 hours of hospitalization, with an overall mortality rate of about 68%. The decreased affinity of β -lactams for Penicillin-Binding Proteins 1A (PBP1A) of *A. baumannii* has been identified as one of its resistance mechanisms. This study utilized a structure-activity relationship pharmacophore approach complemented with molecular docking to screen a library of 12000 phenolics with affinity for PBP1A of *A. baumannii*. Thereafter, the top five phenolics with the most negative docking scores and efficient drug-like features relative to the reference antibiotic (imipenem) were thermodynamically evaluated through a 300-ns molecular dynamics (MD) simulation. Interestingly, the top five leads (ZINC12659587 (–25.20 kcal/mol), ZINC59466627 (–35.01 kcal/mol), ZINC04989868 (–19.67 kcal/mol), ZINC71285349 (–17.90 kcal/mol), and ZINC58836805 (–11.95 kcal/mol)) had better affinity for PBP1A than imipenem (–11.50 kcal/mol) with ZINC59466627 being the most potent of the phenolics. A further probe into the structural architecture of the resulting complexes revealed that PBP1A became more compact and stable following binding the top five phenolics, with reduced amino acid residue fluctuations at its active site. In addition, except for ZINC12659587, quantum calculations using the DFT B3LYP/6-31G+ (dp) revealed that the top five leads are more reactive than imipenem. While the findings from this study lent scientific credence to the profiled phenolics as modulators of PBP1A of *A. baumannii*, further studies using in vitro and in vivo models are underway to validate the potency of the compounds in the management of infections caused by *A. baumannii* [1–3].

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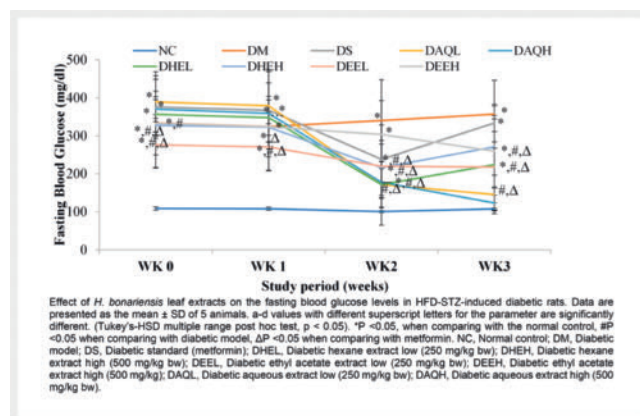
AR3 Hypoglycemic effect of *Hydrocotyle bonariensis* Comm. Ex Lam. leaf extract in an experimental type 2 diabetes rat model

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Hydrocotyle bonariensis Comm. Ex Lam. (Araliaceae) leaf water infusion is traditionally used in the treatment of diabetes mellitus (DM) in Nigeria, Côte d'Ivoire and other parts of Africa [1]. However, studies to validate the claims are not well known and hence, the purpose of the present study. The *H. bonariensis* leaf infusion was prepared and administered at 250 and 500 mg/kg body weight (bw) once daily to high-fat fed streptozotocin-induced diabetes rats for three (3) weeks. α -Amylase and α -glucosidase inhibitory activities were employed to reveal the mechanism of hypoglycemic potential of the extract. Gas chromatography-mass spectrometry (GC-MS) was used to detect the possible phytochemicals of the extract. In addition, *in silico* study based on molecular docking was conducted for possible interaction of the compounds with α -amylase (PDB ID: 1B2Y) and α -glucosidase (PDB ID: 3W37). The results revealed that, *H. bonariensis* leaf water extract showed significant ($p < 0.05$) reduction in fasting blood glucose (FBG) levels (► Fig. 1), ameliorated insulin resistance and improved pancreatic β -cell function compared to diabetic untreated animals and comparable to standard drug. Compounds detected by GC-MS analysis particularly, 1, 2, 3-trimethoxy-5-(1E)-1-propen-1-ylbenzene, thymol and hexadecanoic acid, methyl ester showed a docking score of -5.8, -5.1 and -5.0 kcal/mol with α -glucosidase, respectively. The data confirms the traditional usage of *H. bonariensis* leaf water extract in improving glycemia and recommend further detailed molecular and toxicity studies to support the present data.



► Fig. 1 Effect of *H. bonariensis* leaf water extract on the fasting blood glucose levels in HFD-STZ-induced diabetic rats. Data are presented as the mean $\pm$ SD of 5 animals. * $P < 0.05$, when comparing with the normal control, # $P < 0.05$ when comparing with diabetic model, $\Delta P < 0.05$ when comparing with metformin (Tukey's-HSD multiple range post hoc test, $p < 0.05$). NC, Normal control; DC, Diabetic control; DMF, Diabetic metformin; DWEL, Diabetic water extract low (250 mg/kg bw); DWEH, Diabetic water extract high (500 mg/kg bw); DAQL, Diabetic aqueous extract low (250 mg/kg bw); DAQH, Diabetic aqueous extract high (500 mg/kg bw).

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AR4 A multidisciplinary approach to pharmacognosy: case studies of indigenous South African medicinal plants

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South Africa is one of the most botanically rich countries in the world, harbouring more than 20,000 plant species. A significant portion of these plant species, approximately 4,000 are used as traditional medicine by various cultural groups in South Africa. Research interest in these botanical resources has increased, aiming to promote sustainable cultivation practices and to ensure a consistent supply of high-quality botanicals to produce herbal medicinal products. The slow integration of African Traditional Medicines into the global pharmaceutical mainstream is hindered by lack of sufficient data regarding the quality, safety and efficacy of these herbal products. Over the years, our research has focused on modernising pharmacognosy through the application of novel and complementary analytical techniques for the chemical profiling and quality control of medicinal plants. Through the application of various chromatographic techniques, mass spectrometry, vibrational and imaging spectroscopy, and chemometric data analysis, various challenges related to species identification, delineation, classification, and quality control have been addressed. To support the evidence-based use of traditional medicines, various *in vitro* and *in vivo* models have been employed to generate complementary biological activity data, that validate the traditional use of these plants. In this presentation, a few examples are used to showcase the workflow and subsequent outcomes of using complementary analytical techniques in the phytochemical profiling, and chemotaxonomic differentiation of medicinally important plant species. The application of modern technologies in assessing pharmacological effects, and the subsequent identification of bioactive compounds is also demonstrated.

AR5 Lipid characteristics of sea urchin gonads, in the Bay of Toliara (Madagascar) for their potential use in cosmetics

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This study, conducted from February to July 2024, investigates the lipid potential of the gonads of two sea urchin species, *Tripneustes gratilla* and *Stomopneustes variolaris*, collected from the Great Reef of Toliara (GRT) and Sarodrano, on the southwestern coast of Madagascar. The objective is to develop biotechnological applications while contributing to marine ecosystem conservation by managing the potential impacts of invasive species. The gonads were analyzed in various laboratories, including VALOREMAR at IH.SM, University of Toliara, LME at CNRE, the Division of Phytopharmacy and Pesticide Control at LCP, and the Pharmacodynamics lab at CNARP, where biological tests on cream efficacy were performed. Lipid extraction was carried out using the FOLCH method, yielding 1.56% for *Tripneustes gratilla* and 7.36% for *Stomopneustes variolaris*. Gas chromatography (GC) identified sixteen fatty acid methyl esters in *Stomopneustes variolaris* and eighteen in *Tripneustes gratilla*, based on retention times. The extracted lipids were fractionated using column and thin-layer chromatography, revealing the presence of neutral lipids, apolar lipids, and sterols. All fractions of *Tripneustes gratilla* exhibited positive antioxidant and antimicrobial activity. Acute and skin toxicity tests showed no adverse effects for either

species. A preliminary study on Wistar rats demonstrated relatively rapid hair regrowth following treatment with a cream formulated from *Stomopneustes variolaris* extracts. Anti-inflammatory tests yielded variable results, highlighting promising prospects for future research in marine-derived cosmetic formulations.

AR6 Traditional healing in the desert: Camel urine therapies among communities in southern regions of Morocco

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Camel urine (CU) is emerging as a widely explored traditional remedy, with deep historical roots that date back centuries [1] particularly in southern Morocco, where its therapeutic applications for various illnesses continue to gain momentum in Laayoune-Sakia El Hamra and Guelmim-Oued Noun regions. To better understand this heritage, we conducted a field-based ethnopharmacological study documenting local knowledge and therapeutic customs of 400 traditional practitioners. Our findings reveal that CU is primarily sourced from virgin (46.7%) and pregnant (51.6%) female camels, across various age groups and collected throughout both dry and rainy seasons (57.5%). For treating major health conditions such as cancer, liver disorders, gastric ulcers, diabetes, and tuberculosis, which together represent 69% of reported uses. The typical regimen involves drinking a teacup of fresh urine (70.6%) on an empty stomach (91.1%) once daily (87.1%), usually four times a week (56%). In cases of skin-related ailments, topical use was reported, ranging from one to three teaspoons. Mild adverse effects mostly digestive and transient were noted in 13.8% of cases, with symptoms like nausea, diarrhea, and dry mouth typically lasting just a few hours. These insights confirm that CU continues to play a key role in local medicine and highlight its potential as a source of bioactive compounds for pharmaceutical innovation, skincare formulations, and the development of novel functional food products with promising economic impact.

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AR7 LC- MS/MS approach guided isolation and characterisation of anthraquinone and xanthenes from plant extracts of *Bersama abyssinica*

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Medicinal plants have a rich history in traditional medicine, being used to treat various ailments through a variety of formulations like powders, teas, and infusions. The therapeutic potential of these plants stems from their diverse range of bioactive compounds such as anthraquinone and xanthenes, which are valuable for drug discovery. However, a significant challenge in identifying bioactive compounds is the high cost and time consumption of conventional approach, which often lead to rediscovering previously identified compounds. The computational approaches such as molecular networking is a fruitful approach in drug discovery by rapid screening and identification of bioactive compounds from complex natural product sources.

This work aims to search for active extract/fractions or isolated compounds with antibacterial properties from plant extracts of *Bersama abyssinica*.

The crude extract and fractions showed significant to moderate antibacterial activity with MIC values ranging from 31.2–62.5 µg/mL. The chromatographic techniques based molecular networking led to the identification and characterisation of 07 compounds including 02 xanthenes and 05 anthraquinones among which two unreported. In addition, the use of MS/MS characteristics in comparison with those of analogous structures present within recognised databases enabled the identification of several other compounds. The tested compounds showed no activity.

The molecular networking approach present a fruitful solution in identification and targeting of new compounds from natural plant extracts.

AR8 Traditional Medicine in Transition in Uganda: Past, Present and Future

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Traditional medicine has played a significant role in Uganda's healthcare system, with traditional birth attendants, bone setters, and herbalists being key practitioners. Despite advancements in modern medicine, a considerable portion of the population continues to rely on traditional medicine due to accessibility, cultural beliefs, and affordability [1]. This study explored the role of traditional medicine in Uganda, and how it has been evolving overtime. We assessed the changes in practices, perceptions, and beliefs traditional medicine and how it is integration with conventional medicine in Uganda. The study employed ethnobotanical techniques, including interviews and focus group discussions [2] with practitioners and healthcare professionals, as well as a review of policy documents. We interviewed over 120 practitioners throughout Uganda, representing all regions and a range of practices in the country. The results indicate a gradual shift in the perception of traditional medicine, with increasing efforts to regulate and formalize its practice. While traditional birth attendants have faced restrictions due to maternal health policies, traditional bone setters and herbalists continue to operate, often integrating modern medical knowledge into their practice. The study also highlighted the challenges of regulating traditional medicine, including inadequate documentation, lack of standardized training, and tensions with formal healthcare providers. The research concludes that despite these challenges, traditional medicine remains a crucial aspect of Uganda's healthcare system. Efforts to harmonize traditional and modern practices could enhance patient outcomes and preserve valuable indigenous knowledge. Integrating traditional medicine into Uganda's formal healthcare system, supported by research and policy development, could improve healthcare accessibility and foster collaboration between traditional and modern practitioners.

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AR9 Fish disease pathogens: antibacterial, anti-biofilm and anti-quorum sensing activities of selected medicinal plant extracts

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Fish diseases such as kidney disease, dropsy and vibriosis pose serious challenges to the aquaculture industry, causing significant mortality. Antibacterial, anti-biofilm and anti-quorum sensing properties of selected plant extracts against reference strains and prevalent bacterial pathogens isolated from fishes were investigated. Acetone, ethanol and aqueous leaf extracts of *Alchornea laxiflora* (AL), *Ficus exasperata* (FE), *Morinda lucida* (ML), *Jatropha gossypifolia* (JG), *Ocimum gratissimum* (OG) and *Acalypha wilkesiana* (AW) were screened against fish bacteria (five reference strains: *Citrobacter freundii* ATCC 8090; *Aeromonas hydrophila* ATCC 35654; *Edwardsiella tarda* ATCC 15947; *Pseudomonas fluorescens* ATCC 135225; *Vibrio parahaemolyticus* ATCC 17802; *Vibrio alginolyticus* ATCC 17749; and four clinical isolates: *Lactococcus garviae* B 970/23; *Photobacterium acumselae* B44/16; *Vibrio alginolyticus* B789/2000). Acetone extracts of ML, JG and AW, the ethanol extract of AW, and aqueous extracts of AW and JG all had strong antibacterial activity, each showing excellent minimum inhibitory concentrations (MIC < 0.1 mg/ml) against tested organisms. The reference strains were more resistant than the clinical isolates. The extracts were screened against Vero and Caco-2 cells with tolerable safety margins. The best anti-biofilm (ABF) activity was displayed by ethanolic extracts of OG and AW, aqueous ML extract and ML and FE acetone extracts with ABF > 50% after 24 hours. Most plant extracts had selective minimum quorum sensing inhibition concentrations (MQSICs) against *Chromobacterium violaceum*. These findings highlight a promising alternative to conventional anti-infectives in the form of plant feed additives with potential use in controlling fish pathogens, particularly in terms of their efficacy against biofilm formation.

AR10 Metabolomic landscape of *Helianthus annuus* hybrid seeds feeds the cheminformatic discovery of *K. pneumoniae* MrkH quorum sensing inhibitors

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Sunflower (*Helianthus annuus*) seeds are underexplored, yet phytochemical-rich, with many potential health benefits. *H. annuus*-based metabolites could also be channeled toward the search for novel quorum sensing inhibitors (QSIs), a new class of antimicrobials. Hence, this study modelled sunflower hybrid seeds metabolites against the key QS system protein (MrkH) of *Klebsiella pneumoniae*, a critical priority pathogen requiring novel therapeutics. Following the metabolomic profiling of hybrid seed extracts, *H. annuus* metabolites were modelled against the MrkH protein using advanced computational methods. Molecular docking revealed best binding poses for 5 lead metabolites (calceolarioside A, dicaffeoyl quinic acid, guanosine, cynarin, xanthosine) with a docking score range of -8.8 to -7.8 kcal/mol, relative to the autoinducer (-5.8 kcal/mol). Pharmacokinetics revealed that guanosine and xanthosine are explorable within the Lipinski rule of 5 (LRo5), and other leads beyond the Ro5. Dynamics simulation showed that all leads had higher negative binding free energy (ΔG^{bind}) in the range -42.54 kcal/mol (dicaffeoyl quinic acid) to -28.67 kcal/mol (guanosine) compared to the autoinducer (-10.85 kcal/mol) and cinnamaldehyde standard (-15.88 kcal/mol). All leads had stable interactions with MrkH active site residues (Arg106, Arg107, Asp109, Arg111, Asn183, Gln201, Ser140). Lead ligands-MrkH complexes were thermodynamically stable and compact, with increased number of hydrophobic and hydrogen bond interac-

tions. The lead metabolites from *H. annuus* hybrid seeds could be explored as new MrkH QS system inhibitors after conducting further *in vivo* and *in vitro* validation steps. Sunflower seeds thus hold significant potentials for drug discovery and therapeutics development, among other possible industry applications.

AR11 Cytotoxic, anti-Inflammatory, antioxidant effects of *Cassia abbreviata*, a medicinal plant used in traditional medicine in South Africa

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Medicinal plants have been used as remedies to treat diseases afflicting humanity. Among many diseases treated with plant-based remedies in South Africa include various cancers. The use of traditional medicine has been, in recent years, overshadowed by profound investment in promotion of Western medicine, based mainly on a single compendial active ingredient. Herbal medicine, on the other hand, relies on the combined activities of more than one compound, contributed by more than one species, in herbal preparations. The involvement of more than one active compound in herbal concoctions may be effective, as targeted drug resistance may difficult against a collection of phytochemicals in a mixture. Therefore, this study aimed to demonstrate the anticancer, antioxidant and antiinflammatory activities of *Cassia abbreviata*. Plant leaves were extracted with acetone and tested for their cytotoxic effects against various cancers using the MTT assay, for antioxidant activity using radical scavenging method, antiinflammatory activity, as well as antimetastatic activity using the scratch assay. Moderate antioxidant activity was observed at all tested concentrations. Cell viability reduced to $\pm 60\%$ at 0.5 mg/ml, while displaying a dose-pendent effect on proliferation of MCF7 cells. The extract had antimetastatic effect, inhibiting growth of cells in the wound area. The plant extract reduced ROS activity in LPS-induced RAW 264.7 cells and NO lproduction. The findings of this study strengthen claims of anticancer activities of the plant species. Further studies are underway to ascertain mode of cell death and the pathways through which the plant exerts its biological activities.

Keynote Lectures

KL1 Plant biodiversity in Europe and its conservation

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Europe boasts remarkable plant diversity due to its extensive geographical and ecological gradients, but also the long lasting human transformation. Mountain ranges and islands are a cases of plant diversity, hosting a large amount of endemic and specialised biodiversity. Protecting this biodiversity is a key challenge, as most natural ecosystems face degradation and loss.

With almost no untouched nature remaining in Europe, our approach to conservation is affected.

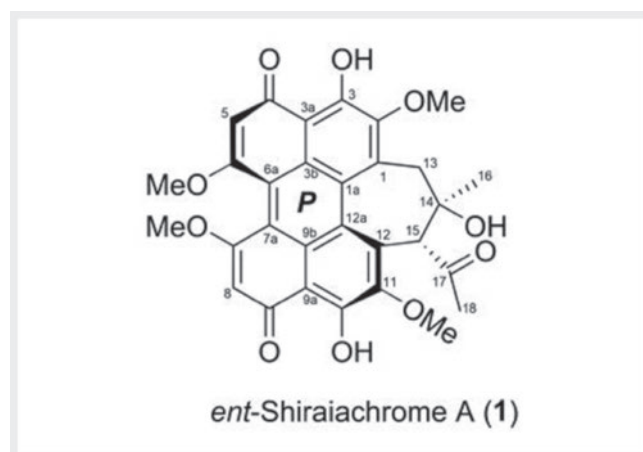
In this lecture I discuss a) the distribution of plant diversity hotspots in Europe and the Mediterranean region, a) the conservation capacity of the Natura 2000 (N2K) network, the world's largest coordinated protected area network established by the EU, and b) the extent of strictly protected areas across EU countries, where natural processes occur without human interference. After having discussed these arguments, I will present some key elements to ensure the long term protection of the European plant diversity.

KL2 Perylenequinones: Fungal metabolites under the spotlight

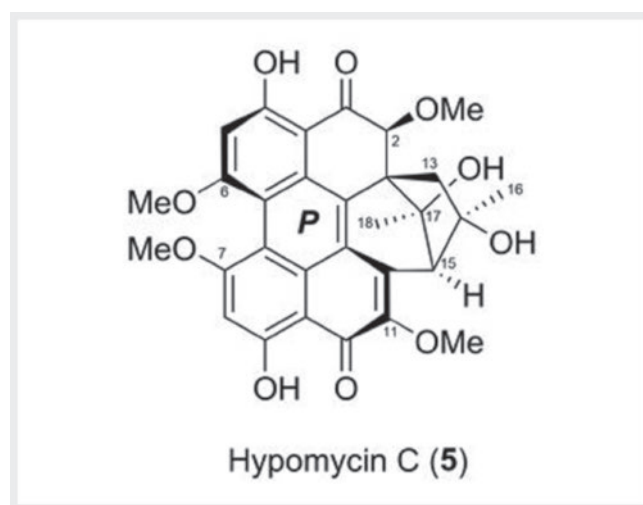
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DOI 10.1055/s-0045-1814813

Fungal metabolites are well known for providing humanity with medical benefits, with penicillin and the antibiotics revolution that it stimulated probably one of the greatest examples. Interestingly, some of these molecules display a high level of conjugation, and as such, can be considered redox active metabolites (i.e., RAMs). With a diverse group of collaborators, we have been studying ways to harness the redox properties of fungal, ► **Fig. 1** perylenequinones, specifically hypocrellins, ► **Fig. 2** [i.e., *ent*-Shiraiachrome A (1)] and hypomyces [i.e., hypomycin C (5)], towards applications in photodynamic therapy and photocatalysis [1, 2]. This talk will present our understanding of these molecules, ranging from fermentation optimization studies to harnessing their redox potential in a variety of applications.



► **Fig. 1** Structure of *ent*-shiraiachrome A (1)



► **Fig. 2** Structure of hypomycin C (5)

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KL3 Medicinal mushrooms – challenges and opportunities

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Medicinal mushrooms are macroscopic fungi, mostly basidiomycetes, which are used in the form of extracts or powder for prevention, alleviation or healing of diseases and/or for completion of our nutrition. Important species are, for example, *Ganoderma lucidum*, Reishi or Ling Zhi, *Lentinula edodes*, Shiitake, *Grifola frondosa*, Maitake, or *Hericium erinaceus*, Lion's Man. The relevant mushroom species are cultivated in large scale and the products are made from the fruiting bodies, the mycelium or the spores. The application of mushrooms for different indications has a very long tradition especially in East Asia and becomes worldwide increasing importance since several years. In Europe, the mushroom products are regulated mostly as dietary supplements and under the food law. Scientific investigations, experiences from traditional medicine and clinical trials show that some fungal species have the potential for the development of regular drugs. To explore this potential, continuous and defined production processes, standardized pharmaceutical quality and methods for its control, preclinical and clinical trials according to the regulations and legal authorization are necessary. In addition to the use of complex fungal preparations, isolated active ingredients from fungi are also of interest as medicinal substances or as lead structures for the development of new drugs.

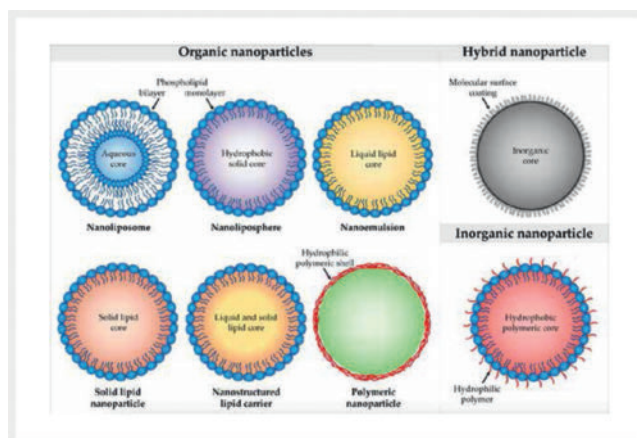
KL4 Unlocking innovations in natural product therapies through nano and micro formulations

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DOI 10.1055/s-0045-1814815

Nutraceuticals offer unique opportunities for preventing disease onset and managing disease progression by leveraging natural agents for oral, dermal, and ophthalmic administration. This keynote will provide a comprehensive view of how the cutting-edge advancements in nano- and micro-formulations could improve the physicochemical properties and biological function of individual molecules. Through the development of innovative nano- and micro-nutraceutical therapies it will be possible to: 1- enhance the stability, solubility, transport, and release of active compounds; 2- synergistically target multiple molecular and cellular pathways through the encapsulation of diverse bioactive agents; 3- optimize bioavailability, transport and absorption by leveraging surface chemistry and biomimetic coatings to improve their passage through biological barriers such as the blood brain barrier (BBB) and intestinal epithelium. Furthermore, recent advancements (► Fig. 1) in lipid-based carriers, polymeric nanoparticles, and hybrid nanostructures enable the protection and precise release of nutraceuticals at the site of action, reducing off-target effects. Stimuli-responsive and smart delivery systems, which respond to pH, temperature, or enzymatic triggers, are emerging as promising approaches to maximize therapeutic potential. Finally, we will spotlight emerging trends in the nutraceutical industry, including the integration of next-generation sequencing, big data, and artificial intelligence to develop personalized solutions tailored to individual genetic, metabolic and microbiome profiles, thereby enhancing efficacy and therapeutic outcomes.



► Fig. 1 Organic, hybrid, and inorganic nanoparticle

KL5 Enhancing and accelerating natural product profiling: The power of CPC, HPLC, HRMS/MS, and NMR synergy

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The inherent chemical complexity of natural extracts poses a significant challenge to the rapid and confident chemical profiling and annotation of bioactive compounds [1]. To tackle this issue, we have developed integrated workflows that leverage the strengths of Centrifugal Partition Chromatography (CPC), High-Performance Liquid Chromatography (HPLC), High-Resolution Tandem Mass Spectrometry (HRMS/MS), and Nuclear Magnetic Resonance (NMR). Together, these methodologies create a synergy that enhances the speed, accuracy, and depth of natural product profiling [2–3].

This keynote will delve into how CPC, as a support-free fractionation technique, is the technique of choice for the chemical deconvolution of natural product mixture while preserving labile compounds, thereby establishing optimal conditions for subsequent spectroscopic analyses. When paired with HPLC and online bioassays, such as DPPH-based antioxidant screening, CPC significantly expedites bioactivity-guided fractionation. HRMS/MS and ¹³C NMR are then employed in conjunction for dereplication, supported by in silico-predicted and curated databases (VersaDB) [4]. These datasets are integrated and visualized using tools like Molecular Networking and heat maps. The various annotations are then classified according to their degree of confidence using the CATHEDRAL tool. Through case studies, we will illustrate the effectiveness of this multimodal strategy in rapidly annotating both major and minor constituents. Special emphasis will be placed on the complementary relationship between MS and NMR data, which enhances structural annotation and fosters the discovery of new bioactive natural products.

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KL6 Bioinformatics, chemoinformatics and FAIR data management in natural products research

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Natural products are a potential source of novel drug leads at the heart of medicinal plant and pharmacognosy research. As analytical techniques—particularly nuclear magnetic resonance (NMR) and mass spectrometry—generate ever-growing datasets, traditional data collection and curation approaches often fall short of the transparency, reusability, and interoperability required for accelerated progress. To address these challenges, we discuss bioinformatics and chemoinformatics approaches to establish FAIR (Findable, Accessible, Interoperable, Reusable) research data management (RDM) solutions tailored to natural product research.

This talk will introduce two complementary, community-driven platforms that illustrate new pathways for FAIR data sharing. nmrXiv (<https://nmrxiv.org>) is an open-access, cloud-based repository designed for NMR data deposition, curation, and analysis, employing open standards and ontology-based annotations to simplify data reuse [1] and foster collaborative research. Alongside nmrXiv, COCONUT—the Collection of Open Natural Products—has evolved into a large-scale, publicly available database that unites extensive chemical and biological metadata on hundreds of thousands of natural products [2]. nmrXiv and COCONUT exemplify how high-quality data can be democratised for the broader scientific community by emphasising open licensing, consistent data structures, and clear curation protocols.

The talk will conclude with examples of research [3–5] enabled by resources such as COCONUT and nmrXiv and discuss how changes in personal and institutional RDM practices embracing collaborative platforms and transparent workflows can lay the groundwork for the next generation of medicinal plant and natural product science.

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KL7 AI-powered literature mining & metabolomics for accelerated molecular exploration

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DOI 10.1055/s-0045-1814818

The exponential growth in metabolomics data and scientific literature creates significant challenges for researchers seeking to extract meaningful insights. The HolobiomicsLab co-developed two complementary AI frameworks that address these barriers: MetaboT and Perspicacité-AI. MetaboT is a multi-agent Large Language Model (LLM) framework that enables natural language-based exploration of mass spectrometry metabolomics data represented as knowledge graphs [1]. This AI-system translates user questions about metabolomics into precise SPARQL queries for knowledge graph exploration, providing answers that cannot be achieved with standalone LLMs. Perspicacité-AI [2] provides an open computational pipeline for literature mining using retrieval-augmented generation and LLM chain-of-thoughts techniques. It transforms bibliographic references into searchable databases and employs an advanced reranking function to deliver precise, source-attributed information from scientific publications. Together, these frameworks democratize AI-access to complex metabolomics data and literature, eliminating technical barriers while maintaining scientific rigor. Our evaluation demonstrates significant improvements in data accessibility and query precision compared to traditional approaches. These open tools enable researchers without specialized programming expertise to discover novel metabolite-species relationships, bioactive compounds, and molecular interactions, accelerating the molecular exploration pipeline across life sciences. In this keynote talk, I will present the concept and performance of these methods and illustrate how they can be used.

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KL8 Volatile fractions and essential oils: Key tools for exploring specialized metabolites in medicinal and aromatic plants

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Plant specialized (secondary) metabolites, especially volatile compounds, are important both for the ecology of plants and for their potential biological activity. Medicinal and aromatic plants rich in bioactive volatile metabolites (referred to as volatile fraction or volatilome) are widely used in the food, cosmetic and health industries. Understanding the chemical composition and biological properties of the volatile fraction is crucial for their safe and effective use.

The volatile fraction can be analyzed using solvent-free headspace sampling techniques, useful for rapid qualitative and quantitative analysis and as an analytical decision maker [1]. However, if biological properties are to be investigated, the volatiles need to be isolated by steam/hydrodistillation to obtain essential oils. These are complex mixtures of odorous compounds, mainly terpenes, which are characterized by high volatility and very low water solubility and exhibit various biological properties, including inhibition of enzymes related to human diseases. Many studies in the literature are often limited to evaluating the overall bioactivity of essential oils without identifying the active compounds.

This lecture investigates the usefulness of volatile fractions and essential oils for the study of plant metabolites and their bioactivity. Sixty essential oils were investigated for their enzymatic inhibition of amylase and cholinesterases, followed by chemical characterization by gas chromatography/mass spectrometry. A bio-guided fractionation approach was used to identify the specific compounds responsible for bioactivity. The results emphasize the importance of selecting the right conditions and enzymatic models [2] for reliable bioactivity data and show how bio-guided fractionation can detect active compounds.

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KL9 Clinical trials of herbal medicines for treatment of common infections: challenges and opportunities

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DOI 10.1055/s-0045-1814820

Antimicrobial resistance is a growing global health challenge, exacerbated by over-use of antibiotics for common infections. Herbal medicines could be recommended as alternatives, if there is sufficient evidence of efficacy.

To review experiences of clinical trials of herbal medicines for common infections, lessons learned, challenges and opportunities for future research.

We have conducted four feasibility trials and one definitive randomised controlled trial of herbal medicines in UK general practice (*Pelargonium sidoides* for acute bronchitis [1]; *Andrographis paniculata* for acute respiratory infections; Shufeng Jiedu (a complex Chinese medicine) for acute exacerbations of COPD) [2], Individualised Chinese medicine for recurrent urinary tract infections [3], and *Arctostaphylos uva-ursi* for acute urinary tract infections [4]. We also conducted public and patient engagement activities to discuss future trials in the UK, China, India, Nigeria and Uganda.

All the trials were able to recruit and retain patients from GP practices, although they faced varying challenges, mainly concerning recruitment and trial procedures. Most patients and GPs were open to trying herbal medicines. Lessons learned include the need to source GMP products which can be taken through to definitive trials, and which patients can easily be advised to purchase. Stakeholders in India, Nigeria and Uganda suggested the need for better clinical trials of local herbal products before these can be recommended in guidelines.

Most patients and doctors in primary care will accept herbal medicines as alternatives to antibiotics for common infections, if there is sufficient clinical evidence. Better clinical trials are needed of available herbs for common infections.

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KL11 The emerging significance of small molecules and marine natural products in immunology and cancer therapy

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Small molecules, organic compounds of low molecular weight, are increasingly recognized for their pivotal role in biomedical research and therapeutic in-

novation. Their ability to precisely modulate biological pathways makes them powerful tools, often offering greater specificity and flexibility than macromolecular approaches such as protein-based drugs or gene therapies. Recent work from our group has focused on chemical immunology and drug discovery, with a particular emphasis on novel small molecule mass spectrometry techniques. Notably, natural products, especially those derived from marine organisms, have emerged as a rich source of structurally unique and biologically potent small molecules. These marine natural products have demonstrated significant potential in cancer therapy by targeting key signaling pathways, overcoming drug resistance, and enhancing immune responses. This presentation will highlight our recent studies exploring the therapeutic relevance of both synthetic and naturally derived small molecules in the context of human disease, with a spotlight on their role in advancing cancer treatment.

KL12 Treasure from garden: anti-obesity and longevity promotion activities of plant natural products

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Obesity presents a significant challenge to the modern society, imposing a substantial burden on health systems and individuals alike. Nowadays, more than a billion people globally are considered obese, a condition that does not solely refer to weight management but rather to mitigating its far-reaching consequences on healthspan and lifespan [1]. Individuals diagnosed with obesity are predisposed to comorbidities including type 2 diabetes, cardiovascular disease, and metabolic complications often referred to as metabolic syndrome. Most importantly, obesity is associated with a reduction in disease-free years, an excess risk of premature death, and accelerated aging [1, 2].

On the other hand, we live in a rapidly aging world, with an expectancy of more than a 2 billion people aged 65 years (or older) by 2050, alongside a rising proportion of age-related diseases. Thus, both obesity and ageing are, *per se*, different sides of the same coin and represent healthcare burden on our society [1, 2]. The development of strategies targeting both of these processes now becomes a challenge for science. Central to this pursuit is the recognition that metabolic health serves as a cornerstone for both healthy weight maintenance and prolonged lifespan [2]. Additionally, key molecular pathways attributed to nutrient signalling that are implicated in obesity progression, intersect with those fundamental to longevity, suggesting potential shared targets for intervention [2].

Utilizing the model organism *Caenorhabditis elegans* (nematode), along with combining omics and molecular pharmacology approaches, our research focuses on the discovery of natural products that target these shared pathways [1–4]. Through our approach, we aim not only to mitigate the adverse effects of obesity but also to uncover novel strategies for promoting healthy aging and longevity.

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Short Lectures

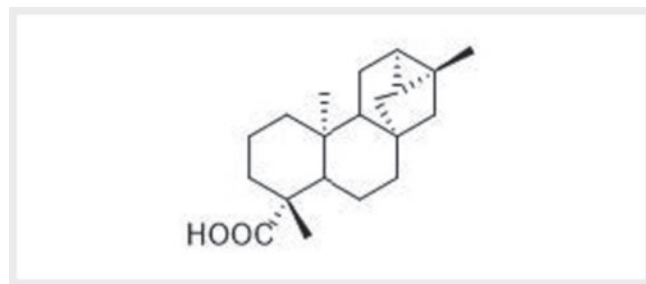
SL1 Studies into the *Croton* species of East Africa – bioactive diterpenoids, community involvement and sustainability

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The *Croton* genus (Euphorbiaceae) is a large and widespread genus with around 1300 species, 32 of which are endemic to East Africa. This genus serves an important role in local communities with different species being used for a wide range of ailments including malaria and fever, bacterial infections and wound healing [1]. The seedcake, husks and oil from *Croton megalocarpus* nuts are of commercial importance being used as animal feed, fertilizer, biofuel, insecticide and for cosmetic applications. As part of a larger project developing sustainability in Kenya, we have investigated the chemical composition and biological activity of *C. megalocarpus*, *C. kinondoensis* and *C. schlefferi* [1–4]. Diterpenoids of the clerodane, cembrane and labdane classes have been identified using standard spectroscopic techniques. The diterpenoids were tested for antibacterial activity against a range of gram-positive and gram-negative bacteria, with trachyloban-19-oic acid (► Fig. 1) inhibiting the growth of drug-resistant strains of *Staphylococcus aureus* and *Enterococcus* spp at a concentration of 25 µg/mL [2]. Clerodanes from *C. schlefferi* increased the proliferation of serum starved dermal fibroblast cells at a concentration of 100 nM [1]. The results would support the potential use of *Croton* spp to treat bacterial infections and wound healing.



► Fig. 1 trachyloban-19-oic acid

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SL2 Development of natural diterpenoid Eriocalyxin B as an anti-tumor agent for breast cancer

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The natural ent-kaurane diterpenoid eriocalyxin B (Eri B), isolated from *Isodon eriocalyx* var. *laxiflora*, has been reported to possess anti-tumor and anti-inflammatory activities. Here, we aimed to illustrate the potent anti-tumor and anti-metastatic effects of Eri B in breast cancer preclinical models. Our previous studies demonstrated that Eri B was an autophagy inducer and could diminish tumor growth in human breast xenograft-bearing mice via inhibition of Akt/mTOR/p70S6K signaling pathway [1]. Eri B was shown to inhibit angiogenesis through modulating VEGFR-2 signaling pathway in zebrafish embryos and breast tumor-bearing mice [2]. Furthermore, our transcriptome analysis in zebrafish embryos revealed two major pathways (glutathione metabolism and tight junctions) which may be related to the anti-tumor activities of Eri B [3]. In our recent studies, the anti-metastatic effects of Eri B have also been demonstrated. Eri B could significantly suppress lung and liver metastasis in both human breast xenograft- and syngeneic breast allograft-bearing mice [4]. While in immune-competent mice treated with Eri B, the diversity and composition of gut microbiome were found to be changed, from which the potential pathways involved in the anti-cancer efficacy of Eri B were projected [4]. Our latest findings revealed the regulatory role of mast cells in breast cancer and the inhibitory effect of Eri B on mast cells in breast cancer [5]. In conclusion, with the promising anti-tumor and anti-metastatic efficacies of Eri B as demonstrated in our breast cancer preclinical models, further investigations on the potential molecular targets and formulation development are currently in progress.

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SL3 Potential use of Propolis-*Punica granatum* mixture as an adjuvant in rheumatoid arthritis therapeutic treatment

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Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by the production of different inflammatory factors [1]. Several therapeutic treatments have been proposed, but their prolonged use produces severe side effects. Furthermore, the complexity of RA pathophysiology prompts to design multi-targeted therapies, effective against the causes and the symptoms of the disease. Therefore, we set up different preparations of propolis-pomegranate peels mixtures (PP) and assayed their anti-inflammatory activity on LPS-stimulated human synoviocytes (SW982) cultured as 2D- and 3D-cell models. The most effective preparation (PP 1:2) induced an evident down-regulation of IL-6 and IL-8 secretion from the stimulated SW982 and modulation of COX-2 and STAT-3, two of the major inflammatory markers, in a dose-dependent manner. The efficacy of PP 1:2 as a potential anti-inflammatory adjuvant was then investigated in an *in vivo* mouse model of collagen-induced arthritis (CIA). Histological analysis of synovial tissues was performed using Masson's trichrome and hematoxylin-and-eosin staining. Additionally, immunohistochemistry was used to assess the infiltration of pro-inflammatory (CD68+) and anti-inflammatory (CD163+) macrophage subsets in the articular tissues. The bioavailability of the main components of PP 1:2 was assessed through LCMS-based pharmacokinetic analyses, which showed that most of the investigated molecules were effectively absorbed. Remarkably, treatment with PP 1:2 at doses of 100 and 150 mg/kg resulted in a significant decrease in mean paw volume of both hind and forelimbs, as well as an improvement in the mean arthritic score. These findings provide novel insights into the potential of PP as a therapeutically valuable natural product for RA.

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SL4 Design and synthesis of a cannabidiol-oleoside hybrid molecules targeting lipoxygenases for inflammation management

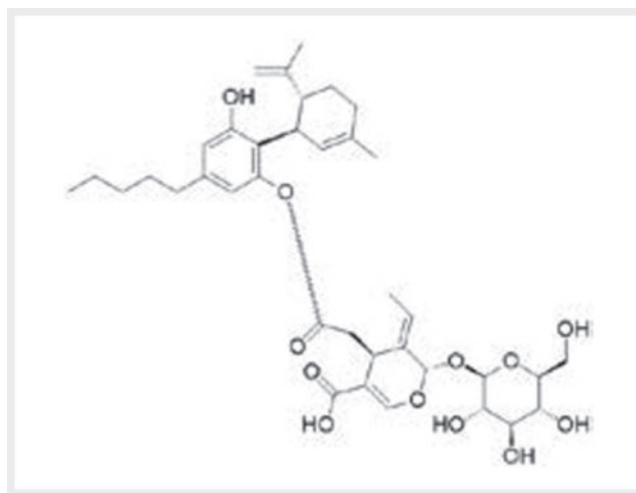
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The chemical coupling of natural bioactive compounds is currently a promising strategy for the design of novel therapeutic agents with enhanced activity. In this study, we designed and synthesized a hybrid compound combining cannabidiol (CBD), a characteristic molecule of *Cannabis sativa* and oleoside, from *Olea europaea*. The focus was to improve the already existing anti-inflammatory efficacy of the two “building blocks” through targeted inhibition of lipoxygenases (LOXs). CBD, a non-psychoactive cannabinoid, is known to inhibit 5-lipoxygenase (5-LOX), the key enzyme in the biosynthesis of pro-inflammatory leukotrienes [1]. Oleoside and its derivative oleacein exhibit anti-inflammatory properties seemingly via cyclooxygenase (COX) inhibition, with indications of additional effects on LOX pathways [2]. First data suggest that the newly developed CBD-oleoside hybrids (► Fig. 1) results in significant 5-LOX

inhibition. Specifically, they show a more potent anti-inflammatory profile, making it a promising candidate for treating 5-LOX-driven diseases, such as asthma, arthritis, Alzheimer and cardiovascular disorders.



► Fig. 1 Basic skeleton of hybrid molecule CBD-Oleoside.

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SL5 Innovative strategies for generating novel bioactive scaffolds from common natural products: Expanding chemical diversity through biotransformation

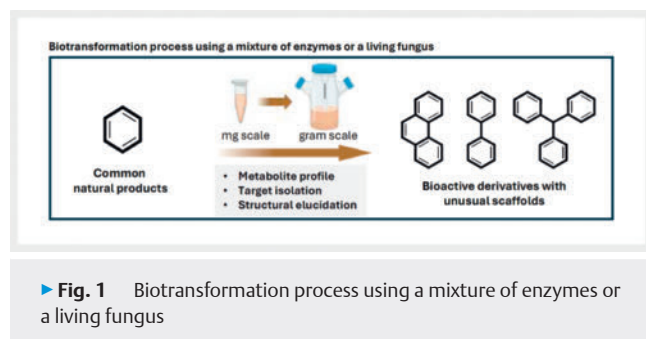
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Over the past decade, natural products (NPs) research has been revolutionized by advances in metabolite profiling, enabling the exploration of previously undetectable bioactive compounds [1]. Despite this progress, plant extracts often remain dominated by common scaffolds with limited value for drug discovery. To overcome this limitation, we explored biotransformation as a strategy to enrich chemical diversity. In this project, we implemented two complementary biotechnological approaches: enzymatic transformation using fungal secretomes (crude mixtures of extracellular enzymes), and whole-cell biotransformation using living saprophytic fungi such as *Botrytis cinerea* and *Trametes versicolor* [2]. Selected substrates included stilbenes, chalcones, phenylpropanoids, and terpenes derived from the Swiss flora. Initial biotransformations were conducted at an analytical scale and monitored using UHPLC-PDA-ELSD-HRMS to detect unusual chemical features. Promising reactions were scale-up at the gram scale, and the resulting products were purified using high-resolution preparative chromatography combined with dry load injection [3]. Enan-

tiomers were further separated by chiral chromatography, and structures were elucidated using HRMS, 2D NMR, and ECD spectroscopy. Compounds were evaluated for their antibacterial and antiviral activity against relevant targets. A library of over 280 compounds was generated at the mg scale. Several exhibited unprecedented scaffolds and potent biological activities [4]. Importantly, mechanistic studies allowed us to propose plausible biosynthetic pathways and identify the enzymes likely involved in their formation [5]. These green chemistry approaches significantly expand the chemical space of NPs, offering new opportunities for drug discovery (► Fig. 1).



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SL6 Biotechnological routes to efficient glycosylation of cannabinoids

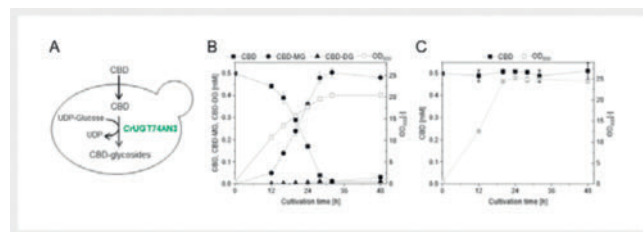
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Phytocannabinoids are a class of natural products with great pharmaceutical potential, but their bioavailability is limited due to their high lipophilicity. Glycosylation is a promising strategy to improve the water solubility and bioavailability of cannabinoids. In this study, the promiscuous UDP-glycosyltransferase CrUGT74AN3 from *Catharanthus roseus* was investigated for its activity towards different cannabinoids (► Fig. 1). In vitro assays, CrUGT74AN3 showed the ability to glycosylate a wide range of cannabinoids, including cannabidiol (CBD), cannabigerol (CBG), and cannabinol (CBN). [1] With the exception of the acidic cannabinoids CBDA and CBGA, glycosides with at least two glucose units were detected for all acceptor molecules tested. To enable the glycosylation of cannabinoids in vivo, a *S. cerevisiae* system expressing CrUGT74AN3 was developed. Optimization of this system by increasing the supply of UDP-glucose through overexpression of phosphoglucosyltransferase (PGM2) and UDP-glucose pyrophosphorylase (UGP1) resulted in a 1.3-fold increase in CBD diglycoside

titer (CBD-DG) and complete conversion of 0.5 mM CBD within 24 hours. Furthermore, a combined in vivo approach using CrUGT74AN3 and the cyclodextrin glycosyltransferase BICGTase from *Bacillus licheniformis* resulted in a further 3-fold increase in CBD diglycoside and CBD glycosides with up to 6 glucose units were produced in vivo. The developed *S. cerevisiae* system is promising to produce cannabinoid glycosides with improved bioavailability. This system provides a basis for further optimization and production of novel cannabinoid glycosides.



► **Fig. 1** Biotransformation of CBD to CBD-monoglycoside (CBD-MG, 1a) and CBD-diglycoside (CBD-DG, 1b) using *S. cerevisiae* cells expressing CrUGT74AN3 (A) Schematic overview (B) Biotransformation experiment using strain yGLY01 expressing CrUGT74AN3, cells were inoculated to an OD600 of 0.5 and 0.5 mM CBD were added to the (C) Control experiment using strain yCS02 containing an empty vector control plasmid; all values are displayed as the mean of three biological replicates; error bars represent standard deviation

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SL7 2D NMR-based biochemometric approach for natural product research: fishing bioactives out of multicomponent mixtures

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Biochemometric approaches [1] using heterocovariance analysis (HetCA) [2, 3] allow the early identification of bioactive compounds from complex mixtures, even if they are minor constituents [3]. HetCA, based on ¹H NMR data has been successfully applied in natural product research [4] and reveals NMR resonances that are positively or negatively correlated with bioactivity [2]. However, a limitation of ¹H NMR is the overlapping of resonances, especially in the aliphatic region. To overcome this drawback, a 2D HSQC-based HetCA approach has been established for an artificial sample set of pentacyclic triterpenes (TTs). Results clearly showed an improved dispersion and visibility of positively and negatively correlated features. The 2D approach was then exemplified on a TT-rich *Eriobotrya japonica* dichloromethane leaf extract (EJD) [5]. The aim was to uncover chemical features of constituents modulating the retinoic acid receptor-related orphan receptor gamma (RORγ) and/or the G-protein coupled bile acid receptor (TGR5). In contrast to 1D HetCA, the 2D pseudospectra allowed to differentiate signals with overlapping ¹H signals and to assess cross-peaks, which are positively correlated to bioactivity, even in the aliphatic region. The 2D NMR approach was able to fish out 2α, 19α-dihydroxy-3-oxo-12-ursen-28-oic acid, a minor compound of EJD (1.98%) with a previously undescribed inverse agonistic activity on RORγ (IC₅₀ 2.15 μM). The results highlight the potential of 2D HetCA as a targeted, rapid and accurate biochemometric tool in the search for novel bioactive compounds from nature [5].

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SL8 Challenges in ligand selection from large virtual libraries: Taking the lion's share of virtual hits

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The emergence of large-scale virtual libraries enriched in natural products (NP), such as LOTUS or COCONUT, has advanced phytochemistry by enhancing both chemical profiling (*i.e.* dereplication) and bioactive compound prioritization *via in silico* drug discovery. [1] However, the generalized nature of these collections—often exceeding hundreds of thousands of structures—raises significant computational bottlenecks in virtual screening workflows (VS), partly due to the absence of targeted filtering. [2] Recent studies have turned to machine learning approaches (ML), to efficiently explore larger chemical spaces without compromising between identifying novel scaffolds and selecting high-scoring compounds. [3] While powerful, these approaches often require substantial data curation, careful parameter tuning, and may face generalization issues beyond the training domain. [4] In contrast, this study provides an alternative strategy to obtain a reduced yet representative subset of ligands from a large NP-focused chemical library. Various molecular descriptors were tested to identify those balancing simplicity and chemical relevance. Among them, the 2D Morgan fingerprint [5] – a type of circular substructure descriptor – proved most effective and accessible. Two methods were then combined to stratify the chemical space: Taylor-Butina clustering was first used to categorize molecules, followed by the Sphere Exclusion algorithm to select representative compounds. [6, 7] Consequently, both chemical novelty (exploration) and representative coverage (exploitation) of the parent library were maintained by selecting a diverse set of compounds, comprising only 20% of the original database. Overall, this approach supports efficient virtual screening, maintaining chemical diversity while reducing computational costs, without requiring machine learning models.

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SL9 Automated NMR spectral profiling of bioactive oligomeric stilbenoids

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Reproducible chemical characterization and validated biological evaluation are key to interdisciplinary natural products (NP) research. To support interdisciplinary reproducibility, the sharing of raw analytical data continues to gain ground in the scientific community and allows researchers to take advantage of the dual qualitative (structural) and quantitative (purity, composition) capabilities of (q) NMR analysis [1]. FAIR sharing of NMR data is particularly useful for rare NPs, a category in which most newly reported compounds fall. In this context, digital spectral data enhance the rigor and reproducibility of the work and are essential for any follow-up studies.

In this context, oligomeric stilbenoids are a prototypical example of structurally diverse and analytically challenging compounds [2]. For such NPs, not least because of their diverse biological activities, the most accurate feasible structural evidence is required to establish valid chemistry-biology relationships. The present study combines the forces of laboratories with a recent track record in newly characterized structures of oligomeric plant phenols, FAIR data sharing of raw NMR data of isolated natural [3] including biotransformed stilbenoids [4, 5], and the development of new methodology that utilizes quantum-mechanical spin analysis (QMSA) to understand even the highly complex ¹H NMR spectra entirely. Our presentation will share the proof of concept for how NMR FID data collections can be transformed into uniquely compound specific digital references (“nuclear genotypes”) that allow both unambiguous identification and purity evaluation of the isolated NPs. The process is amenable to automation and elevates the rigor of characterizing bioactive NPs to a new level.

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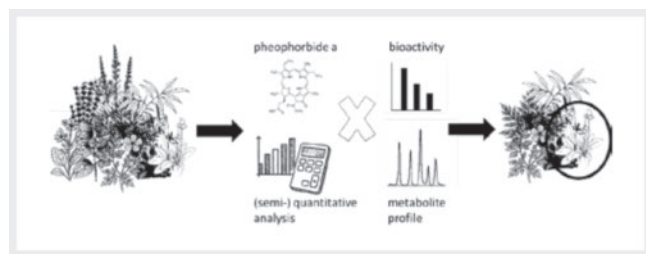
SL10 Targeting the hidden potential: Bioactivity-guided metabolomics for antiviral compound discovery in plants

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The identification of novel bioactive compounds from plant extracts is frequently impeded by the repeated detection of known bioactive molecules – a challenge commonly referred to as dereplication. In the context of antiviral activity, dereplication may be a likely phenomenon with regard to pheophorbide A, a widely occurring chlorophyll degradation product [1]. This compound has the potential to predominate in the results of viral pretreatment assays, leading to redundancy and obscuring the discovery of new antiviral agents (► Fig. 1).



► **Fig. 1** Workflow including correlation of pheophorbide A quantity, protective bioactivity and metabolite profile to prioritize plant extracts.

To address this challenge, we present a strategy that integrates (semi-)quantitative metabolite profiling with bioactivity data to predict and account for the influence of pheophorbide A, thereby streamlining the dereplication process. Using this approach, several plant species were screened and six extracts were prioritized for further fractionation based on bioactivity independent of pheophorbide A. By reducing chemical complexity through data integration, this workflow avoids unnecessary fractionation steps and promotes efficient discovery of bioactive compounds. The method enabled the identification of additional known contributors, such as naringenin, and supported the detection of previously uncharacterized compounds, as demonstrated in *Primula veris* L. Furthermore, this approach powerfully demonstrates how the integration of metabolomics and bioactivity assays enables a more focused and efficient workflow for the discovery of novel agents from complex plant-derived mixtures.

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SL11 Feature-based Molecular Networking in phyllobilin research: New discoveries in *Salvia officinalis*

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DOI 10.1055/s-0045-1814834

Phyllobilins are metabolites of chlorophyll formed during plant senescence, fruit ripening, and post-harvest storage of plant-derived foods. Though discovered over 30 years ago and widespread in nature, phyllobilins have only recently gained attention as a class of bioactive natural products with reported anti-oxidative, anti-inflammatory, and anti-proliferative properties [1]. They are typically identified based on their characteristic UV absorption spectra and fragmentation in tandem-mass spectrometry experiments [2]; however, MS² spectra are still absent from public natural products databases.

This study presents a bioactivity-guided workflow integrating UHPLC-HRMS/MS and feature-based molecular networking (FBMN) to identify phyllobilins and structurally related metabolites. Extracts from *Echinacea purpurea* – a plant known to contain six structurally different phyllobilins – and *Salvia officinalis*, previously unexplored for phyllobilins, were fractionated and analyzed. Using the GNPSFBMN workflow, a molecular network was generated. To establish a reference framework, validated MS² spectra of phyllobilins from *Echinacea* were uploaded to GNPS, enabling annotation of potential phyllobilins in *Salvia officinalis*. These candidate compounds were subsequently isolated and their structures were confirmed via 1D and 2D NMR.

By contributing high-quality phyllobilin MS² spectra to public databases and demonstrating a robust analytical approach, this work facilitates the discovery of novel phyllobilins in crude and complex plant extracts and expands the chemical space of chlorophyll metabolites supporting future studies of their bioactivity and health promoting potential.

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SL12 Metabolic analysis of Triphala polyphenols with gut microbiota-mediated biotransformation by UPLC-PDA-QTOF-MS/MS

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Triphala, a traditional Ayurvedic formulation consisting of a mixture of 3 dried fruits (*Emblia officinalis* L., *Terminalia bellirica* (Gaertn.) Roxb. and *Terminalia chebula* Retz.), is rich in polyphenols and tannins, which contribute to its anti-oxidant, anti-inflammatory and intestinal modulating effects. However, these bioactive compounds exhibit poor oral bioavailability, limiting their direct physiological impact. The gut microbiota plays a crucial role in transforming polyphenols into bioactive metabolites, such as urolithins, which have been associated with improved metabolic and cognitive health. This study aimed to characterize the metabolic profile of Triphala polyphenols upon incubation with INFOGEST digestion and human gut microbiota batch fermentation [1, 2]. Ultra-performance liquid chromatography coupled with diode array detection/quadrupole tandem time-of-flight mass spectrometry (UPLC-PDA-QTOF-MS/

MS) was utilized to identify ellagitannins and their metabolites. The results revealed that urolithins and bile acids were the predominant metabolites. A total of 17 targeted metabolites were identified, exhibiting potent antioxidant and anti-inflammatory properties [3]. Further, a targeted UPLC-MS/MS-based metabolomics approach and colonic fermentation analysis showed that *Triphala* significantly modulated gut microbiota composition, increasing the relative abundance of beneficial *Bacteroides* after 72 h of fermentation. Total phenolic and flavonoid content, along with antioxidant activities (DPPH, ABTS, FRAP, and ORAC), were assessed at 0, 12, 24, and 72 hours of batch fermentation. These findings support the role of polyphenol-derived metabolites in gut homeostasis and microbial diversity. The biotransformation of *Triphala* polyphenols enhances both antioxidant activity and gut microbiota modulation, underscoring its potential as a prebiotic and functional food ingredient. Further studies are warranted to elucidate its long-term health benefits and clinical applications.

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SL13 Fighting inflammation naturally: Anti-inflammatory effects of *Eleutherococcus senticosus* root extract

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Eleutherococcus senticosus (ES) Maxim., commonly known as Siberian ginseng, is a medicinal plant from the *Araliaceae* family traditionally used in Chinese medicine. Rich in flavonoids, triterpenoid saponins, organic acids, phenolic compounds and polysaccharides, ES has shown various biological activities, including antioxidant, anticancer, antifatigue and neuroprotective effects. This study aimed to evaluate the anti-inflammatory activity of a commercial ES root extract, authorized for use in supplements by the Italian Ministerial Decree of 10 August 2018. The extract was tested in allergic and non-allergic *in vivo* murine models of inflammation, including ovalbumin-induced airway sensitization and cerulein-induced acute pancreatitis [1].

In the allergic model, ES treatment (400 mg/Kg) reduced airway hyperresponsiveness, lung tissue damage (H&E staining) and airway remodeling (α -SMA expression). These effects were associated with decreased plasma IgE and pulmonary tryptase levels, indicating reduced mast cell activation. ES showed beneficial effects also in murine pancreatitis. ES administration decreased pancreatic edema and neutrophilic infiltration, as confirmed by pancreatic histology, along with reductions in circulating amylase and arachidonic acid metabolite (PGE₂, PGD₂ and TXB₂) levels.

To explore the mechanisms involved in the protective effects of ES extract, we used an *in vitro* model of acute inflammation, such as lipopolysaccharide

(LPS)-stimulated J774 macrophages. The extract (0.01–1 mg/mL, 2 h before LPS) significantly reduced LPS-induced PGE [2] production, without effects on COX-2 expression. Furthermore, the ES inhibited also nitrite and IL-1 β in LPS-stimulated macrophages.

In conclusion, these findings indicate that ES root extract exerts anti-inflammatory effects *in vitro* and *in vivo*, supporting its potential role in managing inflammation.

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SL14 Comprehensive evaluation of the 'Impact of Cannabinoids' on prostate cancer: Integrating PSA, PET/CT scans, and patient-related outcomes

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Prostate cancer is the fourth most common cancer globally, representing 7.3 % of all cases and 14.1 % of male cancers. This empirical study provides a thorough examination of how cannabinoids impact prostate cancer, covering PSA levels, PET/CT scans, and patient-reported outcomes.

The objectives were to assess the influence of cannabinoids on PSA levels, metabolic activity, tumor size via PET/CT scans, and patient-reported outcomes like pain levels and quality of life.

Using a quantitative approach within a positivist paradigm, participants were recruited from Cancer Care clinics in the Eastern Cape. Inclusion criteria required a prostate cancer diagnosis and specific medical history and cannabis usage adherence. From 125 eligible individuals, 90 participants were determined through sample size calculation. Data collection involved PSA data from pathology reports, PET/CT scan results, and regular patient surveys, utilizing validated tools such as the Brief Pain Inventory and EQ5D questionnaire. Ethical standards were strictly followed, with participants providing voluntary consent. Analysis using SPSS focused on primary objectives related to PSA outcomes and tumor size evaluating PET scans. The secondary objectives included the patient related outcomes such as improved pain relief and enhanced quality of life of patients.

PSA outcomes and tumor size, indicated significant reductions in both among participants using cannabinoids. Notably, median values showed a significant decrease in the group receiving chemotherapy combined with cannabis. An important interaction effect between time and group was observed for the group using cannabis alone compared to the chemotherapy-only group ($p = 0.002$, effect size 0.193).

Likewise, a significant interaction effect between time and group was noted for the group receiving both cannabis and chemotherapy compared to the chemotherapy-only group ($p < 0.001$, effect size 0.324). Patient-reported outcomes also showed improvements in pain management and quality of life. Upon comparing the chemotherapy control group with the cannabis-alone group and the combination group, a remarkably significant disparity in pain intensity scores over time emerged ($p < 0.001$, eta 0.382) and combination group ($p < 0.001$, eta 0.387). The combination group also witnessed a decline in pain scores, especially prominent at the 3-month mark in comparison to the control group. There were notable variances in self-care scores over time between the chemotherapy control group and both the cannabis-alone group ($p < 0.001$, eta 0.490) and the combined cannabis and chemotherapy group ($p < 0.001$, eta 0.628). In both instances, the cannabis and combination groups exhibited a more substantial decrease in self-care scores in comparison to the control group

The study suggests that cannabinoids, whether used alone or with chemotherapy, offer potential benefits for prostate cancer patients, including reducing PSA levels, tumor size, and improving pain management and quality of life. This research contributes to the growing evidence supporting cannabinoids' therapeutic potential in cancer care, with implications for clinical practice and patient-centered approaches. Additionally, insights from the literature review underscore potential synergies between cannabinoids and conventional cancer treatments, particularly in pain management, providing further understanding of their therapeutic mechanisms.

SL15 Unveiling the anticancer potential of *Flaveria trinervia*: Phytochemical screening and GC-MSbased analysis against hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is the most prevalent form of primary liver cancer and a major global health concern. Plants represent a rich source of bioactive compounds [1]. The *Flaveria* genus has been traditionally recognized for its medicinal properties. This study aimed to evaluate the cytotoxic effect of leaf of *Flaveria trinervia* (Spreng) C. Mohr against the HepG2 liver cancer cell line and to identify the phytochemical constituents potentially responsible for these effects. *Flaveria trinervia* leaves (► Fig. 1) were collected, authenticated, dried, and powdered. Phytochemical screening and Gas Chromatography Coupled to Mass Spectrometric (GC-MS) analysis scan mode were performed. Cytotoxic assays were performed on HepG2 and HMEC-1 cell lines using MTT and crystal violet staining [2]. Extract concentrations (0.75–5 µg/µL) were tested. Data were analyzed with ANOVA ($p < 0.01$), and IC₅₀ was calculated with the Hill equation via Quest Graph™. Phytochemical screening of the leaf extract revealed the presence of flavonoids, tannins, saponins, alkaloids, and phenols. GC-MS analysis identified 23 bioactive compounds, including caryophyllene, germacrene D, and phytol, known for their pharmacological potential. Functional assays demonstrated that the extract significantly reduced HepG2 cell viability—by up to 80 % after 24 hours—accompanied by mitochondrial damage, suggesting the activation of apoptotic pathways. The leaf extract of *Flaveria trinervia* exhibits potent cytotoxic and antiproliferative activity against HepG2 cells, likely mediated by its diverse array of bioactive phytochemicals. These findings highlight the therapeutic promise of *Flaveria trinervia* as a natural source of anticancer agents for hepatocellular carcinoma treatment.



► Fig. 1 *Flaveria trinervia* is a species of flowering plant belonging to the Asteraceae family.

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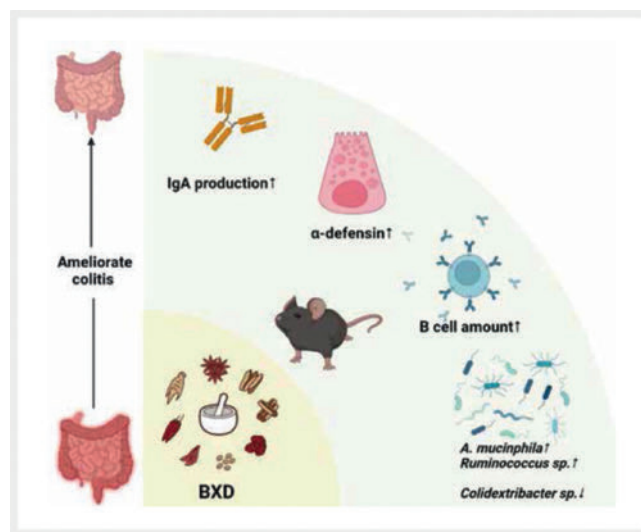
SL16 Banxia Xiexin decoction modulates gut microbiota to ameliorate DSS-induced colitis through increased host defensin and IgA production

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Banxia xiexin decoction (BXD) is a traditional Chinese medicine commonly prescribed for conditions such as ulcerative colitis, gastroenteritis, and diarrhea [1]. Given the role of gut microbiota in immune regulation, we hypothesize that BXD exerts therapeutic effects by modulating microbiota to mitigate colitis. To explore this hypothesis, BXD was administered to a murine model daily for a duration of 14 days. Gut microbiota analysis revealed significant compositional changes, notably an increase in *Akkermansia muciniphila* and *Ruminococcus* sp., and suppression of *Colidextribacter* sp.. Moreover, host RNA sequence of intestinal tissue in treatment group indicated enhanced expression of genes involved in the positive regulation of response to biotic stimulation, including those related to B cell-mediated immune response, immunoglobulin production, and defense response to bacterium. To validate these findings, we performed qRT-PCR analysis and confirmed that BXD treatment enhanced α-defensin (5, 23, 24) expression in the ileum. In the DSS-induced colitis model, BXD pre-treatment significantly reduced weight loss, disease activity, immune cell infiltration, and inflammatory cytokines (IL-1β, IL-6) in colonic tissue, implying its therapeutic value mediated by modulating gut microbiota. To evaluate the effects in the humans, we conducted an intervention study to verify the microbiota changed and IgA production in healthy individuals under treatment. We confirmed the induction of circulating IgA after 14 days treatment and would further analyze the microbiota change in future. In conclusion, BXD pretreatment exhibits preventive efficacy against DSS-induced colitis by inducing changes in gut microbiota composition through enhancing host α-defensin production and IgA production (► Fig. 1).



► Fig. 1 Research workflow

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SL17 Development and characterization of *Moringa oleifera* systems with oligosaccharides: a novel approach to enhance prebiotic and anti-diabetic properties

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Moringa oleifera Lam. is a plant recognized for its significant health-promoting properties, primarily attributed to its high content of polyphenols, which exhibit notable antioxidant and anti-diabetic activities [1]. The present study investigated the ultrasound-assisted extraction of moringa leaves using various solvents to evaluate the prebiotic and anti-diabetic potential of the extracts, alongside the quantification of bioactive compound content. The prebiotic activity was assessed based on the impact of the extracts on selected probiotic strains, namely *Lactocaseibacillus rhamnosus* GG ATCC 53103, *Lactiplantibacillus plantarum* 299v, *Ligilactobacillus salivarius* LA-5, *Lactocaseibacillus casei* Lc-7, and *Lactobacillus helveticus* DSM 20075. Subsequently, a spray-drying technique was employed to obtain homogeneous systems by combining the moringa extract with oligosaccharides (OS), specifically galacto-oligosaccharides (GOS) and chito-oligosaccharides (COS) in three distinct ratios (1:1, 1:2, and 1:4). The resulting formulations (MOR:OS) were standardized for polyphenol content using high-performance liquid chromatography (HPLC). The physicochemical properties of the systems were characterized, differential scanning calorimetry (DSC), thermogravimetric analysis (TG), X-ray diffraction (XRD), as well as density and particle size measurements. *In vitro* analyses were conducted to assess antioxidant activity and the inhibition of α -glucosidase. The release profile of the active ingredients was evaluated at pH levels of 1.2 and 6.8. The findings indicated high polyphenol content in the produced systems, suggesting that the applied extraction and drying techniques preserved the bioactive properties of the compounds. Furthermore, the incorporation of silica mitigated agglomeration, resulting in a more uniform powder. The obtained moringa-OS systems show promise for pharmaceutical and functional food applications due to their preserved bioactivity and improved properties.

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SL18 *Ex vivo* incubation of *Echinacea purpurea* (L.) Moench pressed juice with human gut microbiota

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With the overall aim to investigate the role of gut microbiome for the activity of herbal preparations, dried *Echinacea purpurea* (L.) Moench pressed juice was subjected to *in vitro* upper intestinal tract digestion [1], followed by *ex vivo* incubation using microbiota derived from fecal samples of ten healthy human donors [2].

By UHPLC-HRMS, we identified caffeic acid derivatives, organic acids and alkamides in the initial dried pressed juice and determined a fructofuranoside content of 9 % (calc. as fructose). The overall chemical profile was highly impacted by the gastric phase, but the enhanced levels returned to basal levels or slightly decreased in the intestinal phase of upper intestinal tract digestion. Upon fermentation with fecal samples, the annotated compounds underwent intensive biotransformation, producing metabolites like caffeic acid, 3,4-dihydroxyphenylpropionic acid, and cinnamic acid, which are known from *in vitro* and *in vivo* studies to possess immunomodulatory properties. 16S rRNA sequencing and differential abundance analysis revealed significant modulation of gut microbial taxa, including few, such as *Prevotellaceae* and *Erysipelotrichaceae*, which are associated with immunity and inflammation. Incubation with *E. purpurea* pressed juice also led to significant stimulation of SCFA production and reduction of proteolytic fermentation, while mild donor-dependent immunomodulatory effects were observed in a Caco-2/THP1 co-culture *in vitro* model.

Provided that they also happen *in vivo*, these bidirectional interactions between *E. purpurea* constituents and gut microbiota might contribute to the extract's overall pharmacological effects.

Conflicts of Interest Investigations and MEG were funded by, RB is consultant of and OK is fully employed by Steigerwald Arzneimittelwerk GmbH.

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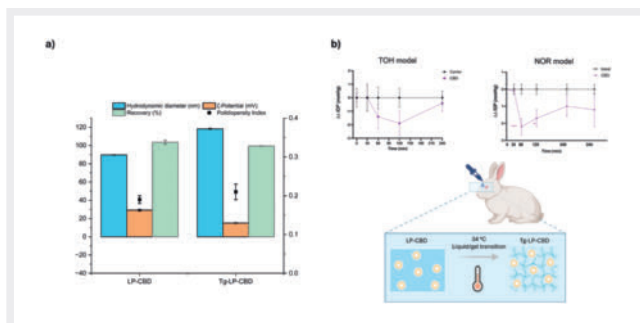
SL19 Thermosensitive chitosan-coated liposome-based gel for ocular delivery of cannabidiol: development, characterization, and *in vivo* evaluation

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Cannabidiol (CBD) is a non-psychoactive phytocannabinoid with known anti-inflammatory and neuroprotective properties. However, its therapeutic potential is limited by poor solubility, low bioavailability, and chemical instability. In this study, we developed an innovative ocular formulation for topical administration of CBD, based on chitosan-coated liposomes (LP-CBD) incorporated into a thermosensitive Poloxamer 407-based gel (Tg-LP-CBD). The lead formulation was optimized using a Quality by Design approach. LP-CBD and Tg-LP-CBD were characterized through TEM imaging, dynamic and electrophoretic light scattering measurements, chemical stability (R %), and mucin interaction studies. The gel exhibited pseudoplastic behavior, appropriate syringeability, and chemical stability for up to four weeks. *In vivo* tolerance was assessed in New Zealand White rabbits over a 7-day period, demonstrating safety for both the empty and CBD-loaded systems at a concentration of 0.05 % w/v. In normotensive and transient ocular hypertension rabbit models, topical administration of 30 μ L of gel LP-CBD significantly reduced intraocular pressure (IOP), with a maximal effect observed between 60 and 120 minutes post-treatment. The CBD-loaded system showed a faster and more pronounced IOP-lowering effect compared to the empty carrier, confirming the pharmacological activity of the formulation [1, 2] (► Fig. 1).



► **Fig. 1** panel a) summarizes the characterization of CBD-loaded liposomes (CBD-LP) and CBD-loaded liposomes by using dynamic and electrophoretic light scattering. (Mean $\pm$ SD, $n = 4$); panel b) illustrates the variation in intraocular pressure ($\Delta\Delta$ IOP, mmHg) following treatment in the transient ocular hypertension (OHT) model induced by intravitreal injection of 5% hypertonic saline solution in rabbits, treated with the CBD-loaded carrier and the empty carrier and changes in intraocular pressure over time (minutes) are normalized to baseline pressure prior to CBD treatment in normotensive (NOR). (Mean $\pm$ SD, $n = 12$) *** $p < 0.0001$ and ** $p < 0.01$ vs baseline.

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SL21 Integrating African traditional medicine into modern healthcare: challenges and opportunities

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Africa harbours a vast and diverse plant biodiversity, with >5,000 plants recognized for their medicinal applications. While some of these medicinal plants have been successfully commercialized, several challenges hinder the advancement of botanical research on the continent. The decline in biodiversity due to overharvesting, deforestation, and climate change; gradual erosion of ethnobotanical knowledge due to urbanization and shifting cultural practices; lack of standardization and quality control procedures; regulatory and ethical complexities in the governance of African traditional medicine (ATMs); insufficient research infrastructure and funding; and the persistent stigmatization of ATMs, often stemming from a perceived lack of scientific validation are some of these challenges.

This presentation highlights key challenges in advancing African medicinal plant research and possible strategic approaches for progress. These include promoting community-led conservation and sustainable harvesting practices, leveraging modern technologies to deepen scientific understanding, and documenting traditional knowledge through collaboration with indigenous communities. Establishing standardized guidelines such as herbal pharmacopoeias is essential, alongside investment in infrastructure and capacity-building

through regional research centers. Strengthening global partnerships, ensuring equitable benefit-sharing, and conducting robust preclinical and clinical studies will enhance the scientific credibility of ATMs. Additionally, raising awareness among policymakers and the public about the value of these botanicals is critical. Achieving meaningful integration of African medicinal plants into global herbal medicine systems and markets will require a unified, multidisciplinary, and collaborative effort.

SL22 A ten-year 2012–2022 retrospective study prevention and treatment of oral mucositis with green propolis mucoadhesive gel

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DOI 10.1055/s-0045-1814844

Oral mucositis is a comorbidity caused by radiotherapy in the head and neck region in cancer patients [1, 2]. Brazilian green propolis has antimicrobial, anti-inflammatory, healing and immunostimulating properties. Retrospective study to evaluate the clinical use of propolis mucoadherent gel (► **Fig. 1**) in the prevention and treatment of oral mucositis. Information was collected from the clinical records of patients seen at the UFMG Faculty of Dentistry Clinic between March 2012 and March 2022. The study was approved by COEP/UFMG (CAAE-0432812.1.0000.5149). MucoadhesiveGel (PharmaNectar®) was indicated for application to the entire oral mucosa 3 times a day (8/8h) [3, 4]. Patients who had no tolerance to propolis were treated with benzydamine hydrochloride or laser therapy. Descriptive analysis was carried out using absolute and percentage frequencies. Patients ($n = 476$) were treated, including men ($n = 343/71.6\%$) and women ($n = 133/28.4\%$), Caucasians ($n = 123/25.84\%$), feodermas ($n = 164/34.41\%$), melanoderms ($n = 154/32.35\%$) and 35 (7.35%) records without this information. The average age was 56 years (± 14 years). Alcoholic patients ($n = 327$), smokers ($n = 336$) and the most prevalent type of tumor was Squamous Cell Carcinoma ($n = 240$). OncOralCare® gel prevent mucositis ($n = 298/32.1\%$) or developed Grade 1 mucositis ($n = 51/10.7\%$) and Grade 2 ($n = 36/7.5\%$). Treatment of Grade 3 ($n = 25/19.0\%$) or Grade 4 ($n = 19/14.5\%$) (► **Fig. 2**) were controlled within 15 days in 90% of cases (► **Fig. 3**). Propolis gel controlled oral mucositis and candidosis. A multicenter, double-blind study will be necessary to confirm these findings.



► **Fig. 1** A-Propolis mucoadhesive gel



► **Fig. 2** Grade 4 oral mucositis



► **Fig. 3** 15 days after treatment with green propolis mucoadhesive gel. Photo: Caldeira, P.C.; Santos, V.R.

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SL23 Skin longevity and dermal health: Analyzing the reshaping action of fenugreek from MAPK pathway to 3D stereophotogrammetry

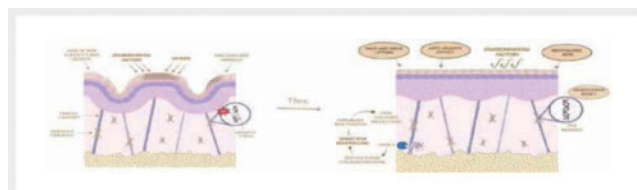
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The cosmetics industry is evolving from a focus on reducing visible signs of ageing to a more holistic and preventive outlook. This has led to the “skin longevity” concept, which aims to maintain healthy, resilient skin by supporting its ability to cope with stress factors [1]. In line with this, we developed a hydrophilic extract from sprouts of *Trigonella foenum-graecum* (Tfex), a plant long used in oriental medicine for its revitalising properties. Tfex, characterised through HPLC-DAD-HESI-MS/MS and HPLC-DAD, is rich in trigonelline (1.13 ± 0.12 mg/g dry extract), apigenin, and vitexin. We assessed its effects on senescence and oxidative stress, focusing on β -galactosidase [2] and SOD (superoxide dismutase), a key antioxidant enzyme [3]. As oxidative stress activates the MAPK pathway and increases MMP-1 expression—leading to collagen I breakdown and ECM disruption—Tfex’s activity suggests a protective effect on dermal integrity [4].

To evaluate clinical efficacy, a randomized, double-blind, placebo-controlled trial was conducted on 42 women (45–65 y.o.) with facial sagging. Over 56 days, treatment with a Tfex-containing emulgel improved hydration, firmness, and collagen content. 3D stereophotogrammetry revealed c-line redefinition and double chin volume reduction, contributing to the restoration of the facial triangle of youth. The reshaping action of Tfex, rich in bioactive molecules, counteracts collagen degradation and damage to the dermis structure. It restores the triangle of youth overcoming the ayurvedic medicine restraints through a scientifically validated protocol, fusing ancient traditions with cutting-edge technologies (► **Fig. 1**).



► **Fig. 1** *Trigonella foenum-graecum* (Tfex) mechanism of action.

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SL24 Unravelling the role of *Pistacia terebinthus* L. in skin aging hallmarks and development of a Cró water-based serum containing microsponges

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Plants' species from the genus *Pistacia* L. (Anacardiaceae) have been traditionally used to treat skin conditions [1, 2]. This ethnopharmacological relevance led the authors to investigate the fruits of *Pistacia terebinthus* L. for potential skin anti-aging applications. Henceforward, hydroethanolic extracts were prepared and characterized through High-performance liquid chromatography-photodiode array detection-electrospray ionization tandem mass spectrometry (HPLC-DAD-ESI/MS), revealing the presence of several gallic acid derivatives and phenylethanoids like pistafolin A. At a non-toxic concentration of 0.4 mg/mL, significant cytoprotective and antioxidant effects ($p < 0.0001$ versus *t*-BHP) were observed in normal human dermal fibroblasts (NHDF). A series of cell-free enzymatic inhibition assays, including the evaluation of anti-hyaluronidase, anti-tyrosinase and anti-elastase effects, showed a pronounced anti-aging potential of *P. terebinthus* extract which were further confirmed by staining the senescence-associated β -galactosidase in etoposide-stimulated NHDF. Moreover, the absence of irritant effects was evaluated through the Test Guideline No. 439 of the Organization for Economic Co-operation and Development (OECD). Building on these outstanding results, the extract was encapsulated into α -cyclodextrin microsponges, to formulate a serum-based on Portuguese Cró thermal water. The serum containing the extract-loaded microsponges showed a low occlusivity factor and an appropriate viscosity. Also, the cell-free antioxidant activity of the extract seems to be enhanced by the presence of Cró thermal water in the serum formulation. Even though, authors warrant further research to confirm the efficacy and safe-

ty of this formulation. Overall, these findings highlight the potential of *P. terebinthus* as a promising natural source of ingredients to develop cosmetic formulations with anti-aging properties.

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SL25 Complex standard formulation against uropathogenic *Escherichia coli* for urinary tract infections: from synergism to improved antibacterial activity and new molecular targets

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For the treatment of urinary tract infections mixtures of different herbal materials are frequently used within traditional clinical practice [1, 2]. A complex formulation, widely used in Germany for preparation of aqueous extracts, with *Betula* sp., *Agropyron repens* (L) P.Beauv., *Solidago gigantea* L. and *Ononis spinosa* L. was infused as a mixture from all four components (combined extract). In addition, the four herbs were extracted separately and the extracts were mixed subsequently (separate extract). None of the extracts influenced proliferation of UPEC-UTI89 and cell viability of T24 bladder cells. The combined extract significantly reduced activity of type-1 fimbriae of UPEC CFT073. This effect was not observed for the mixture of the separately extracted herbs. Systematic investigation of the combined extract and binary mixtures by LC-MS and bioassays indicated that a series of malonylated dammarane triterpenes from *Betula* spp. leaves was extracted in the presence of *Solidago* sp. These dammaranes are responsible for the antiadhesive effect. The combined extract of *Betula* sp. and *S. gigantea* BSC as well as a dammarane-enhanced fraction DEF showed significant antiadhesive effects in a 2D-adhesion assay as well as in 3-dimensional RT4- bladder cell spheroids. RT-qPCR of UTI89 incubated with DEF indicated downregulation of *fimC*, *fimD*, and *fimH* with impact on chaperone-usher-system and correct pili formation. Increased expression of motility gene *fliC* indicates a switch from static to motile life style. S-fimbrial gene *sfaG* was significantly down-regulated, but this did not result in phenotypic changes. Based on an improved extraction of birch leave constituents, data rationalize the importance of combinations of herbal drugs.

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SL26 *Plectranthus* genus: bridging innovation and sustainability in cancer research

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The *Plectranthus* genus, extensively utilized in traditional medicine, represents a rich reservoir of bioactive abietane diterpenoids with significant pharmacological potential [1]. This study investigates key lead compounds derived from *Plectranthus* spp., emphasizing their mechanistic roles in cancer therapy. Among the diterpenoids analyzed, parvifloron D (ParvD) from *P. ecklonii* Benth. and 7 α -acetoxy-6 β -hydroxyroyleanone (Roy) from *P. grandidentatus* Gürke (Lamiaceae) have demonstrated promising bioactivity [1]. A particularly noteworthy derivative, benzoylroyleanone (RoyBz) – a patented compound synthesized from Roy – acts as a PKC-selective activator with potent antiproliferative effects against colon cancer [2]. Mechanistic investigations revealed that RoyBz induces apoptosis via a PKC-dependent mitochondrial pathway, positioning it as a novel therapeutic agent. Functional validation in xenograft mouse models using human colon cancer cells, including PKC δ -knockdown variants, confirmed the essential role of PKC δ in RoyBz-mediated tumor suppression [2]. Furthermore, metabolic analyses uncovered its impact on glucose metabolism, indicating a potential role in metabolic reprogramming, a critical vulnerability in cancer cells [3]. These findings underscore the therapeutic relevance of *Plectranthus*-derived diterpenoids in cancer drug discovery, highlighting RoyBz as a dual-targeting agent capable of disrupting ATP production and reprogramming tumor metabolism [2]. This research reinforces the value of natural products in the development of innovative and sustainable anticancer therapies [1].

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SL27 Revealing the bioactive potential of toad venom extracts through a bio-guided fractionation strategy using centrifugal partition chromatography

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Toad venoms are a rich source of bioactive molecules with various therapeutic properties [1]. This study explores the use of Centrifugal Partition Chromatography (CPC) to extract and purify these compounds, a method previously applied to isolate bufadienolides and alkaloids from toad material. However, few publications exist on this topic, and none describe CPC as a potential method for bio-guided fractionation [2, 3].

The aim was to develop an efficient CPC-based method to isolate active fractions, focusing first on bufadienolides. Methanolic ultrasound-assisted extraction was followed by HPLC-DAD-MS for compound identification. For the purpose of CPC optimization, various solvent systems were tested, starting with the Arizona system (Heptane/AcOEt/MeOH/H₂O, 2/3/2/3), which separated bufadienolides from peptides. However, due to a settling period of several hours, this system required an additional liquid-liquid extraction step to remove peptides, prompting the exploration of ternary solvent systems. The ternary system (Heptane/AcOEt/H₂O, 5/3/2) improved retention but did not achieve complete separation. Adjusting the proportions to 5/1.5/5 eliminated the need

for pre-extraction and isolated two bufadienolides, though polar compounds remained abundant. Using the Arizona system in ascending mode reduced their presence and enabled purification of three bufadienolides.

Biological evaluation was performed on a variety of *in vitro* models. Overall, fractions that contained a polar portion displayed increased activity.

These results show that CPC could provide a scalable method for isolating bufadienolides. Further steps will include additional purification using semi-preparative HPLC.

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SL28 Ebelin lactone enriched lipid-based composition: a novel approach to neurodegeneration management

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Bacopa monnieri (L.) Wettst., known as Brahmi, is a well-known herb from traditional medicinal systems like 'Ayurveda' for its cognitive enhancement properties. It contains bioactive constituents mainly bacosides, which contribute to its therapeutic effects [1]. Despite its diverse pharmacological activities, limited absorption and permeability through the blood-brain barrier restrict its effectiveness, necessitating advancements in extraction and formulation strategies to enhance bioavailability. Ebelin lactone is a hydrolysed product of bacosides and has better activity than bacosides [2]. The current work aims to develop a novel lipidized formulation enriched with ebelin lactone, a carbocyclic triterpene derived from *Bacopa monnieri* extracts through hydrolysis protocol and to enhance systemic bioavailability, and efficacy for managing neurodegeneration, memory deficits, anxiety, and stress, while enabling versatile delivery platforms like capsules, tablets, gummies and more. The herb material has been authenticated and archived in the botanical repository, Pharmanza. From our study the stand-alone ebelin lactone was observed to be potent, however, to optimize the dosages, the lipid-based formulation was developed which demonstrated improved bioavailability through an *in vivo* pharmacokinetic study. Compared to standard extracts, the lipidized composition achieved significantly higher peak plasma concentration (C_{max}), prolonged systemic exposure (AUC), and enhanced delivery of ebelin lactone seen in plasma and brain. These findings support the therapeutic potential of the ingredient for overall cognitive health. In conclusion, the hydrolysed form followed by lipidized formulation of *Bacopa monnieri* extract represents significance in therapeutics by overcoming bioavailability challenges. With enhanced plasma and brain delivery of ebelin lactone, this research work offers promising applications in cognitive health, contributing to the broader use of this important botanical in healthcare.

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SL29 Evaluation of the antibacterial and anti-bio-film activities of essential oils against bovine mastitis causing pathogens

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Bovine mastitis, the inflammation of the mammary gland, is a major concern for the dairy cattle community worldwide. The main mastitis-causing organisms are bacteria, classified as contagious, such as *Staphylococcus aureus*, *Streptococcus dysgalactiae*, and *Streptococcus agalactiae*; and environmental, mainly *Streptococcus spp.* (e.g. *S. uberis*) and coliforms species [1].

Although the development of antibiotic-resistant strains of pathogens has become a critical challenge, the primary treatment of mastitis is still using antibiotics [2]. Thus, looking for alternative therapies, such as essential oils (EO), is necessary [3, 4].

The present *in vitro* study aimed to assess the potential of essential oils as an alternative or complementary treatment to conventional antibiotics against pathogens that cause bovine mastitis.

The bactericidal activity of 11 essential oils and 2 blends was investigated against 5 strains responsible for bovine mastitis: *S. agalactiae*, *S. dysgalactiae*, *S. uberis*, *S. aureus*, and *S. epidermidis*. The assessment of both the reduction of biofilm formation and the inhibition of pre-formed biofilm was carried out against biofilm-formative strains of *S. aureus* and *S. epidermidis*. *Origanum vulgare* and *Cinnamomum zeylanicum* resulted to be the most active essential oils against the above-mentioned bacteria, inhibiting both bacterial growth and biofilm formation.

This research highlights the importance of evaluating natural products to strengthen the hypothesis of safe EO applications for reducing bacterial infections as effective antibiotic alternatives in complementary therapy. Furthermore, combinations of the available antibiotics and natural compounds should also be taken into consideration to avoid treatment failure.

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SL30 Polysaccharides characterization of two seaweeds from the Pacific Northwest and evolution of microbial pathways responsible for their consumption in ruminants

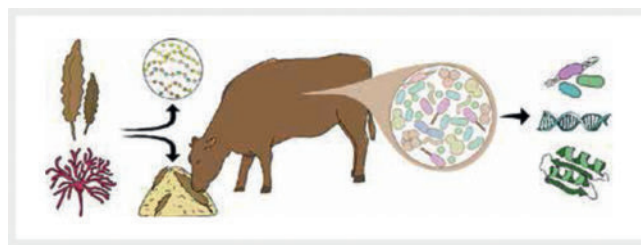
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Seaweed plays a crucial role in carbon cycling and is expected to be a valuable resource for sustainable biomass, with applications in biofuel production, human nutrition, and animal feed. Although seaweed has historically been used as a feed source for livestock grazing near coastlines, the process by which it is digested in the rumen remains unknown. In this study we report the cell wall structures of two seaweeds that were harvested from the waters of the Pacific Northwest, *Saccharina latissima* and *Mazzaella japonica*. To determine how ruminants digest these alga species, we conducted a series of experimental methods, including animal models, bacterial imaging, multilayered meta-omics, bioinformatics, and enzyme biochemistry. We demonstrate that microbial pathways called “polysaccharide utilization loci” dedicated to brown and red seaweed polysaccharide catabolism exist and can be found in geographically distinct ruminants. The evolution of algal consuming intestinal bacteria is complex, and these pathways may have been acquired and maintained through different mechanisms. Algal polysaccharide foraging by the intestinal microbiota appears to be a globally distributed latent trait within the order Ruminantia and carried within microbiome as part of the microbial “dark matter. The maintenance of these pathways within diverse ruminant populations alludes to their role in responding to dietary shifts or the importance of seaweed consumption in the lifestyles of ruminant ancestors (► Fig. 1).



► **Fig. 1** The polysaccharide chemistry of *S. latissima* (brown) and *M. japonica* (red) were characterized by glycomics. Seaweeds were introduced into the diet of ruminants and determined to be metabolized by intestinal bacteria found in the rumen and distal gut. The pathways responsible for metabolism of algal polysaccharides within intestinal bacteria were identified and studied using bacterial imaging, multi-layered meta-omics, bioinformatics and enzyme biochemistry.

SL31 Ethnoveterinary medicine in Africa: assessing the research endeavours from 2001–2024

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Ethnoveterinary medicine (EVM) entails the traditional knowledge and practices used by local communities for the health and productivity of their animals [1]. A systematic literature search was conducted using three prominent multidisciplinary databases: Scopus, Web of Science, and Dimensions, as previously described [3, 4]. We identified 190 relevant documents, revealing an annual growth rate of 5.92 % in publications, with peaks in 2012 and 2022. Citation trends indicate a decline post-2014, reflecting potential shifts in research priorities or ageing foundational works. Journal of Ethnopharmacology, Veterinary Parasitology, and Tropical Animal Health and Production accounted for approximately 40 % of the journal publication. Key contributions include Yiniger (2007) as the most cited document, while Eloff, Masika, and McGaw emerged as leading authors (► **Table 1**). The thematic analysis highlighted a focus on traditional veterinary practices, pharmacological properties of plants, and cultural contexts in livestock health. We established the thematic evolution of Keywords Plus in the field focusing on traditional management of animal health in Africa over the five distinct time periods. Reference spectroscopy traced the earliest citations to 1738, underscoring the historical depth of the field. Institutions such as University of Pretoria and Ahmadu Bello University were identified as EVM research leaders in Africa, with South Africa, Ethiopia, and Nigeria dominating geographical outputs. However, the limited international co-authorship (Belgium and Czech Republic) suggests untapped opportunities for global collaboration. We offer a comprehensive understanding of African EVM research, highlighting its evolution and potential for integrating traditional knowledge into contemporary veterinary practices [2, 5].

► **Table 1** Bibliometric analysis of the most influential authors (H-index ≥ 5) in African ethnoveterinary research and their individual contribution in multi-authored papers

Author	H-index	G-index	M-index	TC	NP	PY_start
ELOFF JN	16	22	0.8	838	22	2005
MASIKA PJ	10	12	0.625	393	12	2009
MCGAW LJ	10	14	0.5	425	14	2005
NAIDOO V	8	9	0.4	327	9	2005
MAPHOSA V	7	7	0.438	210	7	2009
SWAN GE	7	7	0.292	429	7	2001
GITHIORI JB	6	6	0.273	481	6	2003
LULEKAL E	6	8	0.333	569	8	2007
ALOWANOU GG	5	5	0.714	65	5	2018
AZANDO EVB	5	5	0.357	95	5	2011
VAN DAMME P	5	5	0.294	349	5	2008
VAN DER MERWE D	5	5	0.208	470	5	2001

TC = total citation; NP = number of publication; PY_start = starting publication year

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SL32 Phytotherapy in geriatric canine care: managing oxidative stress, inflammation and gut wellness

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Ageing in dogs is a complex phenomenon often associated with chronic inflammation, oxidative stress, and intestinal microbiota changing that can compromise dogs' overall health [1]. The use of phytotherapeutics with antioxidants, anti-inflammatory, and probiotic properties represents a promising approach to improve the quality of life in elderly dogs.

In this study we have tested a mix of *Passiflora*, *Whitania somnifera* and *Taraxacum officinale* in aged dogs, orally administered for 40 days. Blood and serum samples have been collected at the beginning of the experiment (T0), after 20 (T1) and 40 days (T2), and used for biochemical analysis, detection of interleukins by ELISA, and for malondialdehyde (MDA) and Total antioxidant capacity (TAC) assay. Fresh feces have been collected at the same time points to evaluate gut microbiota modification by 16S-rRNA sequencing.

Results revealed that C-reactive protein (CRP) at T0 was significantly higher than T1 and T2 ($p < 0.01$ and $p < 0.001$). Moreover, a decrease in IL-6 and IL-10 cytokines at both T1 ($p < 0.05$) and T2 (0.01) was observed. The mix promoted TAC and reduces lipid peroxidation in the sera of aged dogs, especially at T2 ($p < 0.001$). After treatment, an interesting modulation of the microbiota was observed with a progressive increase from T0 to T2 of lactate-producing bacteria (including *Bifidobacterium* and *Lactobacillus* genera) usually considered as probiotics.

This study demonstrates that the integration of these herbal medicines into dietary or therapeutic regimens could represent a natural and synergistic strategy to promote the general well-being of old dogs.

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SL33 Bioprospecting of understudied mangroves: phytochemical composition and pharmacological potential of *Sonneratia caseolaris* and *Avicennia marina*

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Mangroves thrive in extreme environmental conditions, producing secondary metabolites with significant pharmacological potential, making them a valuable source of novel therapeutic agents. This study aimed to elucidate the phytochemical profiles and evaluate the pharmacological activities of two underexplored mangrove species: *Sonneratia caseolaris* from the Maldives and *Avicennia marina* from the United Arab Emirates. *S. caseolaris* has been used in traditional medicine for its antioxidant, antimicrobial, and antifungal properties, while *A. marina* is recognized for its diverse secondary metabolites with promising pharmacological applications, particularly anticancer potential. The phytochemical profiles of ethanol/water (50 % v/v) extracts from various parts of both species were analyzed using UPLC-ESI/HRMS, and antioxidant potential was assessed using ABTS, DPPH, and ORAC assays. The analyses revealed several compounds newly reported in each species and in mangroves generally. From *S. caseolaris*, 47 compounds were identified, predominantly polyphenols such as phenolic acids, flavonoids, and tannins. The extracts exhibited strong antioxidant activity, with IC₅₀ values comparable to or lower than ascorbic acid.

Similarly, the analysis of *A. marina* identified 49 compounds, including iridoid glycosides, hydroxycinnamic acids, phenylethanoid glycosides, flavonoid glycosides, and triterpene saponins. The propagule pericarp extract displayed the highest antioxidant activity, while the root extract demonstrated cytotoxicity against MDA-MB-231, SW480, and E705 cancer cell lines, with IC₅₀ values of 58.46, 81.98, and 108.10 µg/mL, respectively. In silico analysis identified triterpene saponins as potential contributors to this activity.

These findings enrich the phytochemical understanding of these species and underscore their medicinal potential, supporting further investigation into the unique natural products of mangroves.

SL34 Studies on the isolation and structure elucidation of berberine, hydrastine and jatrorrhizine from goldenseal root (*Hydrastis canadensis* L.) & *Mahonia x media* ‘Winter Sun’ stem bark

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Goldenseal (*Hydrastis canadensis* L., Ranunculaceae) is a traditional North American herbal medicine that has a long history of use for the treatment of a variety of illnesses, primarily as a chemotherapeutic agent for microbial infections. Two of its alkaloid constituents, berberine and hydrastine, have defined pharmacological effects, and research is ongoing. In addition to its presence in

goldenseal, berberine (and analogues) are found in many plant families. One such is the barberry family (Berberidaceae), encompassing various medicinal and decorative species, including those of the genus *Mahonia* (Berberis). This 3-h laboratory experiment has been devised to isolate berberine and hydrastine, from a commercially available root sample of goldenseal, and both berberine and the closely related alkaloid, jatrorrhizine, from the bark of *Mahonia x media* ‘Winter sun’. The isolation procedure is achieved using flash column chromatography on a miniature, sustainable and environmentally friendly scale. A gradient mobile phase system is utilised for the isolation of hydrastine and berberine from goldenseal. The resonance position and degree of splitting of the proton signals of hydrastine is very much influenced by different deuterated solvents. A notable feature of the isolation of berberine and jatrorrhizine from *Mahonia* is the utilisation of silica gel with a 10 % w/w loading of sodium carbonate. In this way jatrorrhizine, an acidic alkaloid, is significantly retained while berberine elutes effortlessly from the column. A follow-on spectral assignment workshop is undertaken using 1- and 2-D NMR, MS and IR spectra obtained on the isolated alkaloids. [1]

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SL35 Protective effects of *Verbascum longipedicellatum* against cigarette smoke-induced inflammation in BEAS-2B cells

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The leaves and flowers of *Verbascum* species, which are used in folk medicine for the treatment of respiratory ailments such as bronchitis, dry cough, and asthma, possess expectorant, antispasmodic, antihistaminic, and antioxidant properties [1–3].

In this study, the cytoprotective activity of *Verbascum longipedicellatum* Hub.-Mor. (Scrophulariaceae) fractions and isolated compounds against cigarette smoke extract (CSE)-induced inflammation on the BEAS-2B cell line was investigated via MTT assay. Then, the protective activity of selected compounds against the inflammatory response and DNA damage induced by CSE were determined via ELISA through IL-6 and 8-OHdG levels, respectively. 50 % methanol extract and *n*-butanol subextract were prepared from the aerial parts of the plant. The VL1E-coded fraction obtained from the *n*-butanol sub-extract using a polyamide column, significantly enhanced the viability of BEAS-2B cells by protecting them against both cytotoxicity and the inflammatory response induced by CSE exposure ($p < 0.05$). Four molecules were isolated from the VL1E fraction; luteolin (1), ethyl-2,4-dihydroxybutanoate (2), 1-O-caffeoyl-β-D-glucopyranose (3), and *p*-anisaldehyde (4). Among the isolated compounds, 1, 3, and 4 demonstrated the highest cytoprotective activity, respectively. In contrast, pre-treatment with 2 resulted in the most substantial reduction in IL-6 levels induced by CSE, followed by 3 and 1. Concurrently, pre-treatment with 2 also led to a notable reduction in oxidative DNA damage in BEAS-2B cells exposed to CSE.

These findings highlight the potential of *V. longipedicellatum*, particularly through fraction VL1E, and compound 2, in mitigating CSE-induced oxidative damage and inflammation, suggesting their promising therapeutic application in preventing cigarette smoke-related cellular damage.

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SL36 Cannabidipholol (CBDP) interactions with Cannabinoid Receptor 1 (CB1R) as a strong negative allosteric modulator

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Building on the 2019 discovery of cannabidipholol (CBDP), a heptyl analogue of CBD isolated from the FM2 chemovar of *Cannabis sativa* [1], and its structural resemblance to known CB1R allosteric modulators [2], this study explored CBDP's potential as a negative allosteric modulator (NAM) of CB1R. Computational modeling pinpointed two likely allosteric binding sites for CBDP, like CBD: the established ORG27569 site and a newly identified intracellular site near TMHs 1, 2, 6, 7, and helix 8. Functional assays and mutagenesis studies confirmed that both compounds interact with this intracellular site, revealing crucial residues for receptor modulation. Importantly, *in vivo* co-administration experiments in mice showed that CBDP effectively counteracted Δ^9 -THC-induced memory impairment while preserving its antinociceptive effects. These results establish CBDP as a novel CB1R NAM targeting an intracellular allosteric site, offering a promising avenue for mitigating the cognitive side effects of Δ^9 -THC in cannabinoid-based therapies.

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SL37 Dual anthelmintic activity of *Cicerbita alpina*

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Anthelmintic resistance is an increasing threat to humans and livestock as the efficacy of established vermifuges continues to decline [1]. In response, we investigated several plant species native to alpine regions with known tradi-

tional uses. The methanol extract of *Cicerbita alpina* (L.) Wallr., a bitter tasting culinary delicacy, showed promising anthelmintic activity [2] and was therefore subjected to in-depth chemical studies.

Liquid-liquid partitioning of the methanolic extract yielded fractions that were tested in parallel in three assays: embryo development (ED), worm motility (WMT) and cytotoxicity. Against *Caenorhabditis elegans*, the polar fractions showed significant inhibition of motility (BuOH: 49% WMT at $c = 150 \mu\text{g/ml}$; EtOAc: 74% WMT at $c = 150 \mu\text{g/ml}$) and simultaneously impaired embryo development [3]. These results suggest that the constituents of the fractions have dual anthelmintic activity over multiple life cycle stages (eggs and adult worms) of helminths. In addition, the BuOH fraction was not cytotoxic to human cell lines. Subsequently, the fractions were purified and single chemical entities were identified that exhibited activities in the range of flubendazole and albendazole. Here we present the identified natural products and their mode of action.

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SL38 Unprecedented and anti-inflammatory secondary metabolites from the African rain forest

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Inflammation is a complex and multifaceted biological response that plays a crucial role in various diseases and conditions, including arthritis, cardiovascular disease, cancer, and neurodegenerative disorders. Despite significant advances in our understanding of inflammation, there remains a pressing need for effective and targeted anti-inflammatory therapies. This study aimed at investigating anti-inflammatory compounds from medicinal plants from the rain forest. *Garcinia smeathmannii* and *Jacaranda mimosifolia* are medicinal plants used for treating and managing intestinal parasites, skin eruptions and skin burns. The crude extract of the leaves of *G. smeathmannii* led to the isolation and identification of twenty compounds (**1–20**) while the investigation of the methanol extract of the flowers of *J. mimosifolia* afforded fourteen secondary metabolites (**21–34**). Two unprecedented compounds including a flavonol (**1**) and a flavone dimer (**2**) were reported. Compound **13** revealed an anti-inflammatory activity which was 9.33 times more active than the reference (Ibuprofen, $\text{IC}_{50} = 11.2 \pm 1.9 \mu\text{g/mL}$) with IC_{50} of $1.2 \pm 0.0 \mu\text{g/mL}$. The same compound **13** was docked on the ovine oCOX-1: meloxicam (PDB Id: 4O1Z) and murine mCOX-2: meloxicam (PDB Id: 4M11) proteins with the docking scores ranging from -10.178 – -6.119 kcal/mol which were in agreement with the experimental anti-inflammatory activity. Chloroflavonin A (**21**) demonstrated potent dual inhibitory effects on Cyclooxygenase-2 (COX-2) and NADPH-Oxidase-5 (NOX-5) enzymes, with binding affinities of -9.7 and -8.2 kcal/mol, respectively, indicating its potential as a strong anti-inflammatory compound. These results provided scientific rationale for the traditional uses of these plants.

SL39 Novel submicron colloidal gels from agro-waste extracts, microalgal polysaccharides and gellan gum: A strategy to produce active coating formulations

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The discovery and formulation of natural products increasingly rely on advanced technologies to identify novel therapeutic and functional properties. This study presents a submicron colloidal gel system combining gellan gum (KF) from *Sphingomonas elodea*, an aqueous polysaccharide extract from *Chlorella vulgaris* (CHLA), and antioxidant-rich polar extracts from hazelnut skins (HS) and chestnut burs (CHES). The aim is to create bioactive formulations for use as natural functional coatings. Differential scanning calorimetry (DSC) identified critical glass transition and gelation temperatures for KF and CHLA, which are essential for stable gel formation. Below these thresholds, colloidal dispersion is incomplete or unstable. When appropriately heated, the system forms submicron-scale dispersions (400–450 nm, DLS) and develops gel networks upon cooling. The presence of divalent cations modulates KF aggregation, promoting higher-order helix conformations and enhancing hydrogen bonding. Interestingly, KF/CHLA gels can form even in the absence of added cations. Scanning Electron Microscopy reveals well-structured, interconnected 3D networks. Rheological analyses demonstrate a clear transition from liquid-like to gel-like behavior. Notably, identifying the optimal inclusion point, during which bioactive extracts are added, ensures their integration without compromising the gel's structural integrity. These findings underscore the rheological and technological properties of the system, establishing it as a flexible, eco-sustainable platform for developing functional coatings and natural delivery systems. The combination of microbial and agrowaste-derived components opens promising avenues for health-related applications [1–3].

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SL40 The power of combining MS and NMR in biochemometrics: Identification of bioactives from *Buddleja officinalis* Maxim. flowers

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The biological testing of natural extracts containing hundreds of constituents has always been a prolific yet challenging approach to identify bioactive natural compounds. In recent years, sophisticated biochemometric tools have emerged as strategy to unravel bioactive constituents from multi-component mixtures. This computer-assisted method is based on the correlation of bioactivity with chemical features. MS- and NMR-based biochemometric approach-

es are both well-established, but the potential of a combined approach is rarely realized. Hence, a combined MS- and NMR-based biochemometric workflow was established to determine the strengths and limitations of both methods in regards to biochemometrical analysis.

For that purpose, a bioactive flower extract of *Buddleja officinalis* Maxim. was selected as illustrative example, previously studied in relation to dry eye disease [1]. The biochemometric analysis was performed according to the established ELINA workflow [2], starting with the microfractionation of the bioactive extract followed by their biological testing as well as recording of their ¹H NMR and LC-MS² data. In addition to the NMR-based analysis already implemented in the ELINA workflow [2], a new and complementary MS-based biochemometric approach integrating molecular networking was developed [3]. The biochemometric results of both approaches were combined streamlining the identification of the bioactive compounds. As a result, the antioxidant and cytotoxic constituents of the investigated extract could unambiguously be unraveled. Therefore, this work highlights the complementarity of MS and NMR facilitating the identification of bioactive natural compounds from multi-component mixtures.

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SL41 Screening natural products for the identification of NRF2 activity modulators

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Aging is a complex biological process caused by the time-dependent loss of cellular homeodynamics and consequently of physiological organismal functions. This process is affected by both genetic and environmental (e.g., diet) factors, as well as by their constant interaction. The balanced functionality of (among others) cellular antioxidant, mitostatic and proteostatic modules is central to genome, mitochondrial and proteome stability. The antioxidant response system comprising the ubiquitously expressed NFE2-related transcription factor 2 (NRF2) and its redox-sensitive cytoplasmic inhibitor Kelch-like ECH-associated protein 1 (KEAP1) defends tissues against (among others) oxidative stress, thereby protecting against pathologies that relate to cellular biomolecules damage. We have shown the dose- and tissue-dependent activity of the NRF2/KEAP1 regulatory network during lifetime, as well as that NRF2 is part of a longevity promoting circuit that ensures functional wiring of proteostatic and mitostatic modules and consequently organismal survival during stress. We have also found a cytoprotective role of NRF2 in various age-related degenerative diseases, as well as that selected natural products can have a positive impact on cellular functionality, also via the activation of the NRF2 pathway, strongly supporting the notion that they can act as potential aging preventive agents and/or therapeutics for age-related disorders. Our rational and experimental platforms (both cell-free and cell-based, as well as at *in vivo* experimental models) for identifying modulators of NRF2 activity with anti-ageing and/or anti-age-related diseases (e.g., cancer, neurodegeneration, etc.) activity, along with some relevant recent findings, will be presented.

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SL42 Novel reactivity-based screening probe for polyacetylenes detection in crude extracts

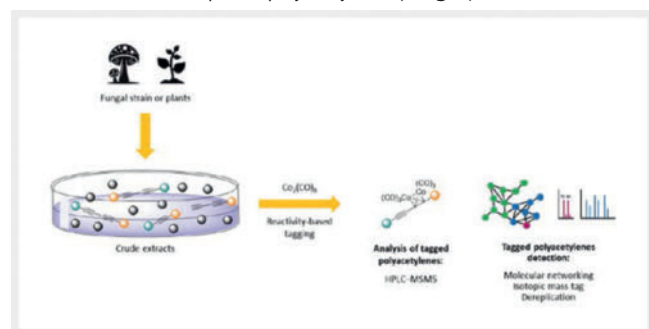
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Natural products are complex structures that play pivotal roles in several biological and ecological processes. Among them, polyacetylenes – produced by both plants and fungi – exhibit antimicrobial and antitumoral activities, making them valuable in food chemistry and crop protection [1]. However, their inherent instability poses significant challenges for isolation and purification. Thus, we recently investigated the reactivity-based approach, involving a reactive azide probe for click chemistry, which led to the discovery of novel triacetylene compounds from *Sistotrema raduloides* [2]. However, internal polyacetylenes aren't easily tagged by azide probes. To address this limitation and enable the selective capture of internal polyacetylenes from crude extracts, we evaluated dicobalt octacarbonyl – a well-known alkyne-protecting agent [3] – as a reactivity-based fishing probe.

Preliminary tests using model diacetylenes demonstrated the successful complexation of internal alkyne moieties. Upon application to a *Daucus carota* crude extract containing three major falcarinol-type internal diacetylenes, feature-based molecular networking (FBMN) revealed successful tagging of the targeted alkynes. However, the detection of all complexed polyacetylenes by FBMN proved to be challenging as successive losses of carbon monoxide (CO) units were observed in the ion source. Nevertheless, these CO losses generated characteristic fragmentation patterns, which could be exploited to rapidly trace the presence of tagged compounds in the MS spectra. This approach validated the dicobalt octacarbonyl probe's efficiency for tagging internal polyacetylenes and revealed novel complexed polyacetylenes (► Fig. 1).



► Fig. 1 Research workflow

Altogether, the combination of a dicobalt-based reactivity probe, FBMN and neutral loss tracking represents a promising strategy for the selective detection of internal polyacetylenes in complex extracts.

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SL43 STW5-II and STW5 modulate common pathways in functional dyspepsia, irritable bowel syndrome and gastroesophageal reflux disease

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The main functional gastrointestinal disorders (FGIDs) include Functional Dyspepsia (FD) and Irritable Bowel Syndrome (IBS) which often show symptoms overlapping with gastroesophageal reflux disease (GERD) [1]. STW5-II, a standardized combination of six plants, is indicated in Germany for the treatment of FD and IBS. STW5, from which it was derived, is also used in FD, IBS and GERD [2].

We analyzed the signaling pathways that are modulated by STW5-II and STW5 and are related to pathomechanisms of all three disease entities to improve our understanding of the modes of action of STW5 / STW5-II as well as of potential joint pathomechanisms of the FGIDs.

We analyzed data (Deep Sequencing, RT-PCR, Microarrays, Elisa, Proteomprofiling) obtained from our previous experiments [3, 4]. Colon cells had been treated with STW5-II and its single constituents (FD, IBS). HET1a cells and rat oesophageal tissue of Sprague Dawley rat had been treated with STW5 and its single constituents (GERD).

The pathways modulated by STW5-II / STW5 in all three models related to immune responses, e.g. T-helper 1 type immune response, cytokine regulations (interferon, interleukin 1, interleukin 2, interleukin 6, interleukin 8, interleukin 10, interleukin 12) and mast cell activity, but also to functions related to muscle activity and transport (cell junction proteins, vasoconstrictions) as well as neural activities. Effects of the single extracts were not additive.

Modulated pathways are related to functional activities that are relevant to treat FD, IBS and GERD. Further analyses are required to understand their interplay as prerequisite for personalized treatment approaches [5].

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SL44 From waste to wealth: Metabolism-driven Isolation and identification of urinary food bioactives

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The discovery of pharmacologically active compounds from natural products (NPs) has traditionally relied on approaches such as bioactivity-guided isolation, biology-oriented approach, or chemistry-oriented [1]. However, these methods often overlook the critical role of metabolic biotransformation in activating NP-derived prodrugs [2]. The metabolism-directed approach (MDM) addresses this gap by emphasizing the functionalization of NPs through human or microbial metabolism, which can generate bioactive derivatives absent in the original source. Many NPs act as prodrugs, requiring enzymatic or gut microbiota modification to yield their pharmacologically active forms. This metabolic activation poses a key challenge for conventional phytochemical studies, as standard bioassays may fail to detect these compounds in crude extracts, rendering traditional isolation strategies ineffective. To overcome these limitations, we present an innovative workflow leveraging urine (and biotechnological alternative sources) as underexplored but rich sources of biologically relevant metabolites [3]. By integrating advanced chromatographic tools from NPs, we demonstrate the first application of continuous flow, liquid-liquid centrifugal techniques i.e. Annular Centrifugal Extraction (ACE) and Centrifugal Partition Chromatography (CPC) for high-yield isolation of FBs metabolic products. Our method combines to achieve efficient, scalable purification of metabolites, followed by accurate structural characterization using high-resolution mass spectrometry (HRMS/MS) and NMR spectroscopy. This approach not only enables the isolation of previously undetectable bioactive compounds but also facilitates biosynthetic pathway mapping and the discovery of biological active metabolites.

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SL45 Conservation and sustainable use of medicinal plants: How do they contribute to the Sustainable Development Goals compliance?

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Conservation and sustainable use of medicinal plants is a “hot topic,” which can have socio-economic impacts. The United Nations (UN) has defined seventeen “Sustainable Development Goals” (SDGs) with the aim of ending poverty, protecting the planet, and ensuring peace. These 17 goals are currently the standard for structuring sustainability measures across sectors and stakeholders. Given that the SDGs are also used in reporting on sustainability in the medicinal plant industry, assessing how the conservation and sustainable use of medicinal plants may contribute to these is necessary [1, 2].

Along the entire value chain, measures potentially impacting the conservation and sustainable use of medicinal plants were captured in a matrix compared to the sustainability goals. Using a Delphi approach, SDGs were ranked according to their influence on them.

Our results show that the SDGs most strongly influenced by the measures were Life on Land (SDG15), Responsible Consumption and Production (SDG12), and Quality Education (SDG4) in ranks 1, 2, and 3, respectively. This is in line with the type of these measures, including wild harvesting, agricultural production, processing, and quality control of medicinal plants.

Our approach has indicated the SDGs that are most significantly influenced by measures taken, e.g., their inclusion in the sustainability reports of medicinal plant industries. However, initiatives cannot focus on specific SDGs, as a sustainable approach in the field needs also to address other SDGs, e.g., Gender equality (SDG5) or Peace, justice and strong institutions (SDG16), to build the basis of a bright future for the use of medicinal plants.

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SL46 Samabriva: a plant-based biotechnology company for high value molecules production

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Samabriva is a plant-based biotechnology company offering an innovative, flexible bioproduction platform already used in the pharmaceutical and cosmetics industries. Our system combines the benefits of plant-based methods—low cost, safety, and serum- and animal-free processes—with the scalability of traditional bioreactors. This enables continuous, cost-effective, and sustainable production of high-value molecules year-round, anywhere in the world.

Today, secondary metabolites are primarily sourced from field-grown plants, yielding only minimal quantities. Current methods lack scalable, local, and sustainable alternatives. Samabriva’s proprietary hairy root-based technology provides a novel approach, significantly enhancing production efficiency.

We have developed strong expertise in genetic engineering and process optimization, achieving over a 10,000-fold increase in metabolite productivity compared to open-field cultivation. Additionally, 95 % of the target metabolite is secreted into the culture medium, simplifying extraction and reducing environmental impact. Our technology enables industrial-scale cultivation of hairy root clones in bioreactors, facilitating local production of valuable natural compounds.

To further streamline the pipeline from seed to optimized clone, we apply design of experiment (DoE) strategies across the process. Current efforts include optimizing *Rhizobium rhizogenes*-mediated infection to increase throughput and reduce project turnaround times. This approach is especially valuable for rare or endangered species where plant material is limited.

SL47 Comprehensive chemical profiling of eco-sustainable Extracts from “Cavolfiore della Piana del Sele” PGI (*Brassica oleracea* L. var. *botrytis*) inner leaves by LC-HRMS- and NMR Metabolomics

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Cauliflower (*Brassica oleracea* var. *botrytis*), a member of the Brassicaceae family, is cultivated worldwide for its edible head, which consists of undeveloped flower buds. In Italy, cauliflower is extensively grown, and some regions have specific varieties recognized for their unique qualities [1]. Recently, Cavolfiore (cauliflower) della Piana del Sele has been registered by the European Union with the Protected Geographical Indication (PGI) quality label [2].

The inner and outer leaves are a significant byproduct during the processing of cauliflower, typically accounting for around 40–50% of the total plant biomass [3]. In the frame of a project aimed at investigating plant wastes as source of bioactives in a circular economy perspective, a study was conducted on the inner leaves of Cavolfiore della Piana del Sele PGI. An unconventional method, Microwave-Assisted Extraction (MAE), was chosen to prepare eco-sustainable extracts using EtOH and a 1:1 EtOH:H₂O mixture as solvents. To obtain comprehensive information on the primary and specialized metabolites of the cauliflower leaves, a combined LC-ESI/HRMS and ¹H NMR metabolomics approach was developed. The LC-ESI/HRMS profile allowed the identification of primary metabolites belonging to various classes of polar lipids, as well as specialized metabolites including glucosinolates, flavonoids, and phenylpropanoids. Additionally, ¹H NMR spectra of the extracts revealed the presence of organic acids, sugars, and amino acids. The relative concentration of metabolites identified by ¹H NMR was determined in comparison with the known concentration of the internal standard (TSP), using Chenomx software, which enables the quantification of individual components in NMR spectra.

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SL48 The fate of chlorophyll in hops: Profiling phyllobilins in senescent leaves of *Humulus lupulus* L.

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Hops (*Humulus lupulus* L., Cannabaceae) have long been valued for their role in brewing and traditional medicine, primarily due to their content of bitter acids and volatile compounds [1, 2]. Large portions of hop biomass, however, particularly senescent leaves, remain underutilized despite their potential as sources of phytochemicals. This study aimed to investigate bilin-type chlorophyll metabolites — phyllobilins (PBs) — in hop leaves. PBs are increasingly recognized as bioactive molecules with antioxidant, anti-inflammatory, and anti-cancer properties [3].

In our study, we profiled senescent hop leaves using chromatographic and spectroscopic techniques, revealing the presence of diverse PBs including di-oxobilin-type phylloleucobilins (DPLEBs) and phylloxanthobilins (DPxBs). DPxBs are yellow pigments, which we show to contribute to the coloration of hop leaves in autumn. The identified DPLEBs and DPxBs from hops displayed a characteristic structural feature that could hint towards a diversified chlorophyll catabolism. Our findings suggest that hop leaves, typically discarded post-harvest, constitute a promising and sustainable source of bioactive PBs. This work expands the phytochemical profile of *H. lupulus* and underscores its potential for nutraceutical and phytotherapeutic applications beyond traditional uses.

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SL49 Seasonal variation, enrichment and geo-accumulation of trace and heavy metals in superficial sediments along urbanised Austral wetlands in Mpumalanga

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Urban wetlands get polluted by heavy metals being discharged from industries, urbanisation and agriculture, which tend to alter the sediment quality [1]. Sediments are important as they act as a sink for nutrients in aquatic environments. Present study aimed to assess the spatiotemporal distribution and sediment pollution indices of trace and heavy metals in superficial sediments and identify potential sources of contamination in urbanised Austral wetlands. Sediment samples were collected within the Riverside's Wetlands, City of Nelspruit, South Africa, across three seasons (i.e., hot-dry, hot-wet and cool-dry seasons). A multivariate principle component analysis (PCA) was used to determine the metal relationship. The metal pollution indices, such as contamination factor, enrichment factor, geo-accumulation factor and pollution load index were used to determine the pollution of metals in the sediments across different sites among seasons. The study showed that among all metal dynamics, only Na concentration was lower during cool-dry season than other metals. The PCA showed that except Na, all metals in wetlands sediment were highly correlated with each other, signifying a strong positive association. The pollution indices indicated that wetland sediments are highly contaminated, mainly with Mn, Co, Cu, Pb, Zn, Al and Fe, especially during the cool-dry season. The current study highlights the urgent need to make great efforts to control the industrial emission and the exceptionally severe heavy metal pollution to the wetlands,

and the immediate measures should be carried out to minimize the rate of contamination and extent of future metal contamination.

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SL50 Exploitation of microwave hydrodiffusion and gravity extraction to produce health-promoting juice and nutraceuticals from pomegranate (*Punica granatum* L.)

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Pomegranate (*Punica granatum* L.) juice (PJ) consumption has recently gained popularity worldwide for preventing cardiovascular and metabolic disease [1]. Among its health-promoting compounds, ellagitannins, anthocyanidins, flavan-3-ols, and phenolic acids are the main representatives [2]. However, the preparation of PJ currently involves industrial practices which can modify its physico-chemical characteristics while reducing its beneficial effects. In this scenario, the first aim of this study was to use microwave hydrodiffusion and gravity as an alternative and green technology to produce PJ from fruits of the Wonderful cultivar.

The microwave power (MP) and extraction time (ET) that yielded the highest PJ output, phenolic content, and antioxidant activity were identified through a central composite design study. The best conditions resulted in a MP of 1.3 W/min and an ET of 40 min, also producing a PJ with the best anti-glycation and anti-glucosidase activities highlighting its use in the nutraceutical industries.

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SL51 Comprehensive analysis of phenolic compounds from *Solanum glaucophyllum* Desf. including the new glaucophylloside

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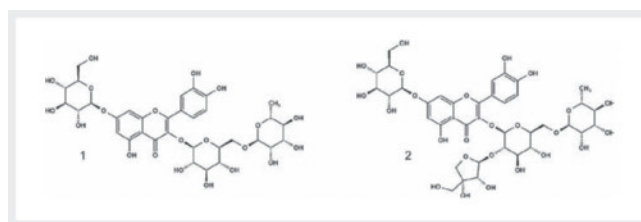
DOI 10.1055/s-0045-1814873

Although *Solanum glaucophyllum* Desf. (SG) has been in the focus of scientific interest since the late 1960s, mainly due to its known calcinogenic properties, the plant has been very little studied from a phytochemical point of view. Actually, only 8 phenolic glycosides, such as avicularin, quercetin-rutinoside and quercetin-apiosyl-rutinoside, have been described in the literature. [1] The aim was therefore to comprehensively isolate, identify and quantify phenolic compounds.

First, a crude extract was prepared from dried leaf material. With different reversed-phase chromatography systems 3 novel compounds for SG were isolated.

These were unequivocally identified by HR-MS and 2D-NMR as 3/4-O-p-coumaroylglucaric acid, 3/4-O-caffeoylglucaric acid and 3-O-caffeoylquinic acid. LC-MS/MS experiments led to the detection of a total of 16 glucaric acid and 6 quinic acid derivatives. These were subsequently quantitated using commercial reference standards or isolated pure substances in MRM mode.

Furthermore, a total of 5 quercetin derivatives was isolated from the crude extract. The structures of two novel compounds were elucidated after permethylation of the target fraction and subsequent separation by normal phase chromatography. 7-O- β -glucosyl-rutin (1) was novel in SG, while 7-O- β -glucosyl-apiosyl-rutin (Glaucophylloside, 2) (► Fig. 1) was described for the first time in literature.



► Fig. 1 Structure of 7-O-beta-glucosyl-rutin (1) and glaucophylloside (2)

The independent synthesis of authentic reference material (1-O-caffeoyl-, 3-O-caffeoyl- and 3-O-feruloyl- β -glucoside) led to the identification and quantification of 5 cinnamic acid glucosides.

In summary, 33 phenolic compounds were quantitated in SG and 29 of these substances were detected in this plant for the first time. Thus, the phytochemical spectrum of SG was considerably expanded with this work. [2]

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SL52 Biological assessment and phytochemical investigation of commercial bean varieties by NMR and LC-ESI-HR-MS analysis

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The common bean (*Phaseolus vulgaris* L.) is a major dietary source of protein, complex carbohydrates, dietary fiber and polyunsaturated fatty acids [1]. The polar lipid profile and polyphenolic content of five bean varieties that are often consumed in Italy (Cannellino (PVCA), Controne (PVCO), Borlotti (PVBO), Stregoni (PVST), and Vellutina (PVVE)) were examined in this study using a metabolomic approach combining NMR and LC-MS analysis. Using high-resolution mass spectrometry and MS/MS fragmentation, along with database searches and literature references on *P. vulgaris*, a total of 33 polar lipids, such as fatty acids, phospholipids, oxylipins, and N-acyl glycerolipid and 27 polyphenolic metabolites, including phenolic acids, flavonoids, catechins, anthocyanins, and saponins, were identified. The beans' mineral content was also assessed. Principal component analysis (PCA) of the extracts revealed significant compositional differences, particularly for PVBO. Given the wide range of bioactive metabolites identified, the

vitro inhibitory activity of the seed extracts against cyclooxygenase-2 (COX-2), was performed. Among all, PVCO exhibited the strongest COX-2 inhibition, with an IC_{50} value of $31.15 \pm 2.16 \mu\text{g/mL}$. Based on these findings, PVCO's anti-inflammatory potential was further evaluated in LPS-stimulated murine macrophage cells. At a concentration of $30 \mu\text{g/mL}$, PVCO significantly reduced TNF- α release, although its effect on IL-6 secretion was less pronounced. In conclusion, this is the first study to report PVCO's potential anti-inflammatory properties, and it demonstrates how LC-MS and NMR may work together to offer thorough insights into food composition, assisting efforts to preserve food biodiversity and track the origin of agricultural products [2, 3].

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SL53 Importance of complementary analysis for revealing the identity of unknown newpsychoactive substance: experiment of the UNODC exercise

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The problem of drug abuse and misuse (whether natural or synthetic) is universal, and almost every country is dealing with it differently. In particular, the emergence of newpsychoactive substances (NPS) is a global concern, especially as these molecules vary and are continuously revolutionised by perpetrators. Furthermore, their health effects might be ambiguous and could lead to serious harm or even death. Consequently, their detection requires highly skilled expertise and through using modern analytical instruments. In this regard, the United Nations Office of Drugs and Crime (UNODC) regularly organises an international exercise through which a hypothesised NPS is chemically synthesised where participating laboratories are asked to reveal the NPS identity. Importantly, literature data about this unknown molecule is unavailable and participants are expected to use any data as a guide for their quest. During the 2024 exercise, we participated in this activity, and we were able to determine the identity of this theoretical molecule using multiple instruments. In this presentation, we demonstrate our strategy for identifying the unknown compound using NMR, LC-MS, FT-IR and elemental analysis. Moreover, we discuss some pitfalls that we encountered during this experiment. Finally, it is crucial for such analysis to implement a complementary analysis of instruments which can compile the scattered information into a complete structure for the hypothesised molecule.

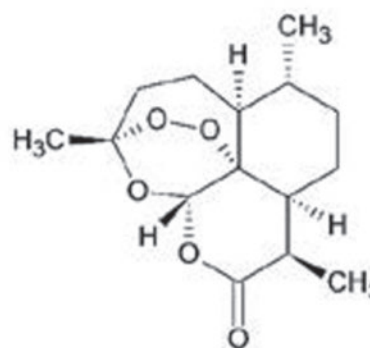
SL54 Multi-omics approaches to reveal lipid bioprospecting potential in marine coastal sponges

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Terpenoids represent one of the largest classes of secondary metabolites or natural products. They are widely used as relevant pharmaceuticals, particularly in cancer therapy and in the prevention and treatment of inflammatory diseases. Porifera (sponges) are an important source of novel marine natural products and due to the large number of species need preliminary screening which leads to the significance of dereplication strategy. Here, C18 reversed-phase solid-phase extraction (RPSPE) methodologies has been used for the discovery of terpenoids and related lipid compounds. Also, mass-spectrometry-based lipidomics has been applied to characterize fractions with different classes of terpenoids.



► **Fig. 1** Chemical structure of antimalarial sesquiterpene Artemisinin

Freeze-dried sponges ($n = 32$ in total) sampled from Irish coastal waters were extracted and fractionated by reverse phase solid phase extraction (1 g C18 stationary phase) into 4 fractions: H_2O , $\text{H}_2\text{O}/\text{MeOH}$ (1:1, v/v), MeOH, MeOH/DCM (1:1) [1]. A portion (1 mg) of fraction MeOH and MeOH/DCM (1:1) were screened by GC-MS after derivatization and HPLC. Based on GC-MS results, the major lipids produced are fatty acids, including characteristic 'demosponic acids' (delta-5,9 unsaturated long chain fatty acids) and diverse sterols, likely the polyhydroxy sterols as reported [2, 3]. Screening analysis also highlighted the presence of terpenoids in *Dysidea fragilis*. UHPLC-HR(+)ESI MS analysis suggests the presence of sesquiterpenoids, particularly two isobaric sesquiterpenoids [4]. Upscaling extraction and isolation will be implemented on targeted sponges for the isolation and structure elucidation of bioactive lipids.

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SL55 Identification of hazardous compounds in food matrices through integrated biological and chemical approaches focused on High-Performance Thin-Layer Chromatography

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Food and food-related products may contain hazardous compounds from raw materials, processing, or packaging, introducing safety uncertainties in the food chain. Identifying toxicologically relevant chemicals (e.g., genotoxic/endocrine disruptors) requires efficient screening using integrated approaches [1]. The identification and characterization of potential hazards in extracts using traditional bioassays exhibits some limitations [2]. Therefore, approaches including fit-for-purpose bioassays for an efficient screening have been promoted. For this, a procedure combining high-performance thinlayer chromatography (HPTLC) bioautography (p-YES/Umu-C assay) was developed [3, 4]. Thus, a method combining high-performance thin-layer chromatography (HPTLC) bioautography (p-YES/Umu-C assay) with high-resolution mass spectrometry (HRMS) and microfractionation enables targeted isolation of bioactive compounds. Traditional TLC-MS desorption is a valuable tool however, is limited by resolution and metabolite yield, whereas HRMS-based metabolite profiling of crude extracts—paired with HPTLC bioactivity-guided fractionation—preserves sensitive bioactivity while improving chromatographic resolution. This workflow allows MS dereplication of known compounds and upscaling to HPLC semi-prep with dry load injection for targeted isolation in a single step [5]. Building on the success of the conclusive application to soy-based ingredients, the methodology was further extended to encompass other food-related complex matrices. Finally, the effectiveness of integrated approaches in contributing to the identification of potential hazardous compounds is promising.

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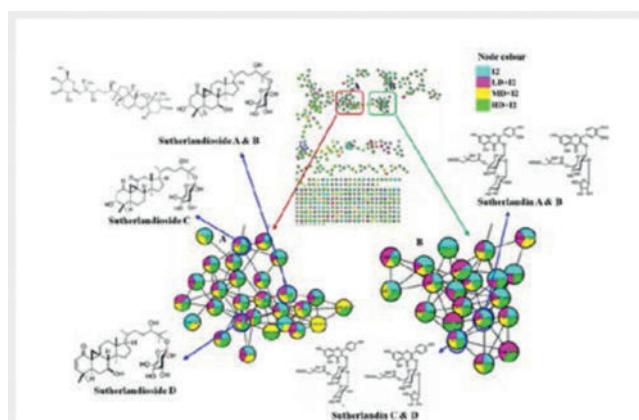
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SL56 Untargeted metabolomics for evaluating the effects of inoculation on *Lessertia frutescens* under stress

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Lessertia frutescens is a multipurpose medicinal plant indigenous to South Africa [1, 2]. The demand for *L. frutescens* raw materials has increased due to its anti-HIV [3], anti-inflammatory [4], anticancer [2] and antioxidant [1] properties, putting undue pressure on wild populations. Commercial propagation of the plant presents a feasible alternative to supplement the dwindling supply of wild populations, but the persistent drought climate poses a threat to growth and productivity of the plant. Rhizobia can improve yield, biological activities and the production of secondary metabolites plants under drought. The aim of this work was to evaluate the efficacy of three rhizobia isolates, TUTLFNC33,

TUTLFNC37 and TUTLFWC74 trapped using a homologous host *L. frutescens* from rhizosphere soils sampled in the Western Cape and Northern Cape, South Africa to mitigate climate induced stress. The rhizobial isolates were used to inoculate *L. frutescens* seedlings under low, moderate and extreme drought. Metabolomic profiles were obtained using a Shimadzu UHPLC-qTOF-MS, followed by annotation and obtaining molecular networks of *L. frutescens* metabolites. Isolate TUTLFNC37 improved the drought tolerance through root proliferation and proline accumulation. Increased biomass, phenolic compounds, triterpenes and antioxidant activity, were evident in the extracts of *Lessertia frutescens* inoculated with TUTLFNC37. Metabolomics molecular network demonstrated the ability of the rhizobial isolate to withstand drastic changes in the composition of unique metabolites, sutherlandiosides A-D and sutherlandins A-D. Peptides and glycerolipids molecular families, which were never reported to be present in *Lessertia frutescens* before, were detected and annotated in the molecular networks (► Fig. 1). Therefore, isolate TUTLFNC37 is regarded the most promising, environmentally friendly intervention to enhance drought tolerance of *L. frutescens*.



► Fig. 1 Molecular network showing metabolite families in *L. frutescens* extracts inoculated with TUTLFNC37 after exposure to different drought stress levels. Each node colour represents a drought stress level to which the plants were subjected.

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SL57 NMR Metabolomics to elucidate mode of action of Traditional Chinese Medicine

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Traditional Chinese Medicine (TCM), practised alongside Western Medicine, has been a significant part of the healthcare system. [1] Their complex chemical composition leads to an incomplete mode of action (MoA) study. In this project, we aim to use NMR metabolomics to elucidate the MoA of TCMs tra-

ditionally used for treating Parkinson's disease (PD). Mitochondrial dysfunction is one of the causes of PD. [2, 3] 6-hydroxydopamine (6-OHDA), a mitochondrial inhibitor, has been widely used to create a cellular model for PD research. [4, 5] As a proof-of-concept study, the metabolome of 6-OHDA-treated SH-SY5Y was analysed to understand and elucidate the MoA of 6-OHDA. Our results demonstrated reactive oxygen species (ROS) induced oxidative stress and electron transport chain disruption, consistent with the existing 6-OHDA toxicity. This study aided in establishing and optimising the experimental methods. Further, 6-OHDA-treated SH-SY5Y cells were subjected to a cell viability assay to evaluate the protective effects of TCM *Puerariae lobatae* Radix, *Platycladi semen* and *Rehmanniae praeparata* Radix. Specifically, NMR metabolomics was employed to evaluate the changes in cellular metabolites when treated with 6-OHDA in the presence of TCM extracts, shedding light on how the TCM could protect cells from 6-OHDA toxicity. Our results will help determine whether NMR metabolomics is a useful tool for TCM MoA study. The extracts of these TCMs were also subjected to chemical analysis using chromatography, mass spectrometry and 1-dimensional and 2-dimensional NMR. Flavonoids and their glycosides were purified from *Puerariae lobatae*; fatty acids were identified from *Platycladi semen*, and furanic compounds were purified from *Rehmanniae praeparata*.

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SL58 Mushroom characterization and identification: No one method, fits all!

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High Performance Thin-Layer Chromatography (HPTLC) is a well-established analytical technique widely utilized for botanical and fungal identification, especially when sensitivity and specificity are built into each analysis for mushroom characterization and identification purposes. Mushrooms are known for their potentially 'bioactive' compounds (beta-glucans, sterols, and triterpenoids, etc.) that may have a wide range of human health benefits, such as enhancing immune system function, enhanced antioxidant activity, and anti-inflammatory effects, among others. [1–4] Method selection for mushroom identity, frequently varies with species of mushroom, fungal part, compound(s) of interest, form of material, as well as the type of extract, etc. In this presentation we aim to demonstrate how mushrooms can be identified by HPTLC, using multiple 'fingerprints', as needed, to differentiate one species and its individual fungal parts, and commercial mixtures. HPLC, LC/MS/MS quantitative data will also be reported for distinguishing fruiting body from mycelia for various species, LOD in mixtures, and to quantitate and verify the HPTLC findings. Over 15 different mushroom species for both mycelia and fruiting body were analyzed and studied, utilizing three or more HPTLC methods. Data from several commercially popular mushroom species will be presented. We find that there is no 'one method, fits all', for mushroom characterization and identification, or for any botanical, with both sensitivity and specificity, adequately considered. We demonstrate how the power of any method can be enhanced orthogonally, and the identification certainty of the qualitative analytical process, increased.

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SL59 Fungal transformations of medium-chain fatty acids into commercially valuable methyl ketones with flavor and fragrance properties

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Methyl ketones are highly valuable fatty acid derivatives with flavor and fragrance properties mainly used as food aroma compounds, insecticides, pheromones, biofuels and chemical intermediates [1–3]. They occur in nature, however due to their low concentration, methyl ketones are synthesized via chemical oxidation of hydrocarbons. In our research, 47 whole cell fungi, among them 18 endophytes, were employed in the transformations of medium-chain fatty acids (octanoic, decanoic, dodecanoic and tetradecanoic acids) into their respective methyl ketones (2-heptanone, 2-nonanone, 2-undecanone and 2-tridecanone). Based on the screening experiments, selected fungi showing promising biocatalytic activity were successfully chosen for preparative scale (1 g/L) biotransformations. The isolation yields of 59% for 2-heptanone, 75% for 2-nonanone and 45% for 2-undecanone afforded by corresponding *Penicillium roqueforti*, *Penicillium corylophilum* and *Trichoderma viride* make the biocatalytic processes an effective alternative to chemically synthesized methyl ketones influencing on reduction of environmental impact. The research is part of the ongoing INBIOWAL project which focuses, among others, on the application of biocatalytic processes to transform lipids isolated from oil industry waste, such as gums and oleins, into high-value products for industrial use.

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SL60 Heptelidic acid oligomers from the fungal strain MSX83315

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Fungal-derived sesquiterpenes represent a unique category of secondary metabolites that distinguishes them from other natural sources. Sesquiterpenoid oligomers are a special type of natural product that consists of at least two linked sesquiterpenoid units. Numerous biosynthetic reactions occur throughout the oligomerization process, including oxidation, chlorination, hydrolysis, and etc. Theoretically, the large number of permutations on the building blocks makes this class worthy of further exploration. Due to our interest in the chemical diversity of fungi, four previously undescribed heptelidic acid derived oligomeric sesquiterpenes, including two trimeric and two tetrameric sesquiterpenes, along with 13 known monomeric, dimeric, and trimeric sesquiterpenes, were isolated from fungal strain MSX83315. Structures, inclusive of absolute configurations, were assigned by detailed spectroscopic analysis, ECD calculations, and biosynthetic considerations. The structural diversity generated by this fungal strain was interesting, as few trimeric and tetrameric sesquiterpenes have been reported from Nature.

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SL61 Targeted isolation of antifungal natural products from endophytic fungi of alpine plants through multi-informative metabolite networks

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Endophytes, including fungi and bacteria, are groups of microorganisms that protect their hosts against biotic stresses such as pathogen attack while promoting growth and enhancing nutrient uptake [1]. In the extreme alpine environment—characterized by long winters, intense UV exposure, and an elevated prevalence of phytopathogens in recent years—these microorganisms are thought to produce specialized secondary metabolites that support plant survival. To discover novel antifungal natural products from endophytic fungi of alpine plants against one of the most destructive agricultural pathogens, *Botrytis cinerea*, we isolated over thirty fungal species from more than ten plants growing in the Alps, including *Picea abies* and *Gentiana clusii*. Following molecular identification, ethyl acetate extracts from these fungi were screened for bioactivity against *B. cinerea*, and their metabolic profiling were simultaneously carried out by untargeted LC-HRMS/MS, in combination with Feature-Based Molecular Networking (FBMN) and SIRIUS [2, 3] for metabolite annotation. Integrating bioactivity and metabolomics data into a multi-informative molecular network enabled prioritization of bioactive scaffolds for targeted isolation. This approach identified three fungal strains exhibiting potent antifungal activity (MIC < 100 µg/mL). Followed by large-scale fermentation, over twenty secondary metabolites, including chlorinated chromophilones and novel cytochalasin derivatives were isolated. Among these, three compounds with MIC values below 10 µg/mL were characterized. Structure of the compounds isolated was elucidated using 1 and 2D NMR, and their absolute configuration were determined by electronic circular dichroism. This study highlights an integrated workflow for the discovery of novel antifungal compounds from alpine endophytes, for targeting agriculturally important phytopathogens.

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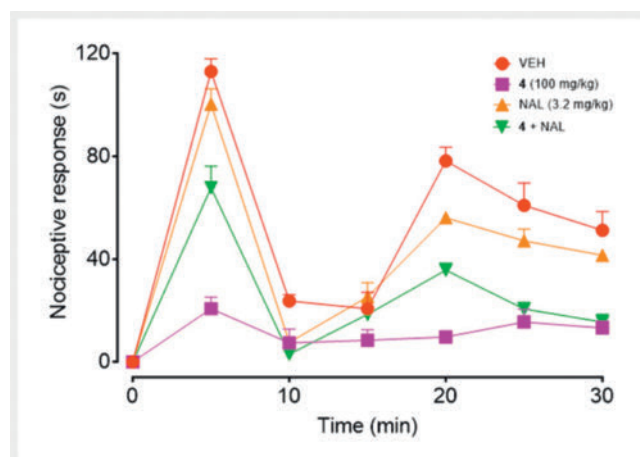
SL62 Chemistry and antinociceptive activity of the traditional preparation and compounds from *Ageratina grandifolia* (Regel) King & H. Rob. (Asteraceae)

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The traditional preparation and major compounds [3,5-diprenyl-4-hydroxyacetophenone (1), *O*-methylencecalinol (2), encecalin (3), and encecalinol (4)] of *Ageratina grandifolia* [1], tested at doses of 10, 31.6, and 100 mg/kg, administered intragastrically, demonstrated a significant antinociceptive effect in both phases of the formalin test in mice. Encecalinol (4) was the most active compound, exhibiting a dose-dependent antinociceptive action in both phases (► Fig. 1); its effect was substantially reduced when the mice were pretreated with naloxone. Additionally, four new chemical entities were identified, including 2-hydroxy- α -truxillic acid (5), (3*R*,4*S*)-2,2-dimethyl-3-hydroxy-4-(1-angeloyloxy)-6-acetyl-7-methoxychromane (6), *N*-tricosanoyltyramine (7), and grandifolamide (8). The volatile constituents of *A. grandifolia* were characterized using gas chromatography-mass spectrometry analysis of the components obtained through head-space solid microextraction and hydrodistillation. The volatile compounds contained a high percentage of chromenes, caryophyllene, germacrene D, spathulenol, and caryophyllene oxide. A suitable liquid chromatographic method was developed and validated to simultaneously quantify three significant flavonoids in the ethyl acetate soluble fraction of the traditional preparation, specifically 6-hydroxykaempferol-7-*O*- β -D-glucopyranoside, quercetagenin-7-*O*-[6-*O*-caffeoyl- β -D-glucopyranoside, and 6-hydroxykaempferol-7-*O*-[6-*O*-caffeoyl- β -D-glucopyranoside].



► **Fig. 1** Antinociceptive effect of encecalinol (4) at 100 mg/kg (p.o.) in combination with naloxone (NAL; 3.2 mg/kg, p.o.) using the formalin test in mice.

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SL63 Redox-regenerative natural products: An innovative approach to ferroptosis inhibition and redox balance maintenance

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Ferroptosis is a regulated cell death mechanism driven by iron-dependent accumulation of lipid peroxides [1, 2]. It is widely recognized as a critical component in the progression of devastating conditions, including neurodegenerative disorders, degenerative liver diseases, and ischemic organ damage [3]. In chronic degenerative diseases, prolonged metabolic imbalance in iron, lipid, and redox pathways promotes a gradual buildup of oxidative damage, driving cells toward ferroptosis. While lipophilic antioxidants such as liproxstatin-1 offer rapid protection against ferroptosis in acute events, their long-term use in chronic conditions risks disrupting cellular signaling and homeostasis [4]. Our research aims to develop strategies to counteract ferroptosis and restore metabolic balance. Through bioactivity-guided isolation of extracts from 19 traditional Vietnamese medicinal plants, we identified two biogenic agents as potent inhibitors of ferroptosis in human HepaRG liver cells, with EC₅₀ values of 10 nM and 0.3 µM. Remarkably, the more potent agent outperformed the well-known endogenous ferroptosis inhibitor vitamin E by a factor of 10. Both natural products share a common mechanism: inhibiting lipid peroxidation and redox-regeneration through the ferroptosis suppressor FSP1. Utilising enzymatic redox cycling, these agents offer a distinct advantage over traditional antioxidants by maintaining prolonged intracellular activity through continuous regeneration, while potentially selectively targeting lipid peroxidation without disrupting essential redox signaling. Notably, both compounds mitigated toxicity in a *Caenorhabditis elegans* ferroptosis model, highlighting their potential *in vivo* efficacy.

In conclusion, our study identifies two redox-regenerative biogenic agents as promising, innovative anti-ferroptotic leads, offering a novel approach to prevent pathological cell death in degenerative diseases.

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SL64 Phytochemical characterization of oleogum resins from *Commiphora* species of socotra island

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Socotra (Yemen), a large island in the Indian Ocean, is a biodiversity hotspot known for its unique flora and fauna. Species belonging to the genus *Commiphora* Jacq. (Burseraceae) stands out, as it includes resin-producing plants valued for their phytochemical richness [1]. Despite the island's ecological value, the oleogum resins of *Commiphora* species from Socotra remain poorly

studied. This research investigates resins from Socotran taxa (*C. kua* (R.Br. ex Royle) Vollesen, *C. ornifolia* (Balf.f.) J.B.Gillett, *C. planifrons* Engl., *C. parvifolia* Engl., and *C. socotrana* Engl.) aiming to explore their phytochemical profiles. Volatile compounds were analyzed using headspace solid-phase microextraction coupled with gas chromatography-mass spectrometry (HS-SPME-GC-MS) [2], while non-volatile constituents were studied using high-performance liquid chromatography coupled with high resolution mass spectrometry (HPLC-HRMS), high-performance thin-layer chromatography (HPTLC), and nuclear magnetic resonance (NMR) spectroscopy. The volatile fractions are rich in monoterpenes and sesquiterpenes, with chemotaxonomic significance. In the non-volatile fraction, we identified lignans, including yangambin, a furofuran lignan known for its vasorelaxant and anti-inflammatory properties [3, 4]. Yangambin, previously reported in *Commiphora wightii* (Arn.) Bhandari [5] is found here for the first time in Socotran species. This finding expands the known chemical diversity of the genus and suggests that these plants may produce bioactive compounds of pharmaceutical interest. The occurrence of lignans in the resins highlights the potential of Socotran *Commiphora* species as novel sources of secondary metabolites with possible pharmacological applications. Further studies will aim to isolate and characterize additional compounds, and to evaluate their biological activities.

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SL65 Phytochemicals from *Terminalia brownii* and *Acacia asak* as natural inhibitors of SARS-CoV-2 main protease: In Vitro, molecular docking, and simulation analysis

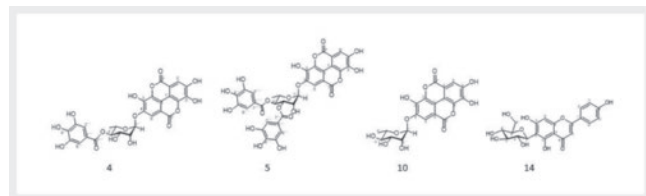
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The continuous emergence of SARS-CoV-2 variants and the absence of a definitive cure underscore the need for effective antiviral agents. In this study, the alcoholic extracts of *Terminalia brownii* and *Acacia asak*—among 18 Saudi medicinal plants screened—showed the highest inhibitory activity against the SARS-CoV-2 main protease (Mpro) [1], with inhibition rates of 95.3 % and 95.2 %, respectively, at 100 µg/mL. A bioassay-guided fractionation of the active *n*-butanol extract from *T. brownii* yielded eleven compounds, including phenolic acids, hydrolysable tannins, and one flavonoid. Additionally, four flavonoids were isolated from *A. asak*. Structural elucidation of all compounds was performed using spectroscopic methods and literature comparison. Molecular docking studies using AutoDock 4.2 identified compounds **4**, **5**, **10** (from *T. brownii*), and **14** (from *A. asak*) (► Fig. 1) as promising Mpro inhibitors, with

binding energies of −9.3, −8.5, −8.1, and −7.8 kcal/mol, respectively. These interactions were further validated through 100 ns molecular dynamics simulations, assessing RMSD, RMSF, Rg, and SASA values. In vitro assays confirmed the inhibitory activity of the four compounds against Mpro, with compound 4 showing the most potent activity ($IC_{50} = 1.2 \mu\text{g/mL}$). MM-GBSA binding free energy calculations supported these findings, highlighting compound 4 as the strongest binder. Importantly, none of the tested compounds exhibited significant cytotoxicity against A549 and HUVEC cell lines, suggesting favorable safety profiles.



► **Fig. 1** Chemical structures of the isolated active compounds.

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SL66 Beta-terpineol from citrus essential oil modulated dipeptidyl peptidase-IV towards type 2 diabetes care: An experimental- and molecular dynamics simulation-based study

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Despite the reported antidiabetic potential of citrus essential oils (CEOs), their inhibitory effect on dipeptidyl peptidase 4 (DPP-IV) remains elusive. This study investigated the anti-DPP-IV potential of six citrus species (*Citrus aurantifolia*, *Citrus bergamia*, *Citrus limon*, *Citrus paradisi*, *Citrus reticulata*, and *Citrus sinensis*) essential oils using experimental and computational approaches. *In vitro* analysis revealed that the six CEOs exhibited significant inhibitory effect on DPP-IV with *C. reticulata* being the most potent ($IC_{50} = 8.00 \mu\text{g/mL}$) relative to the effect observed with the reference standard, sitagliptin ($IC_{50} = 196.40 \mu\text{g/mL}$). Bioactive constituent identification produced a library of 64 metabolites while the top ranked metabolites produced good pharmacokinetic properties and remarkably safety. Molecular dynamics simulation study of the top five metabolites revealed beta-terpineol as having the best affinity (binding free energy: −35.78 kcal/mol) for DPP-IV compared to sitagliptin (−34.80 kcal/mol). While the resulting complexes from the other top-ranked metabolites were relatively stable, further structural analysis positioned beta-terpineol as having superior binding interaction, stability, hydrogen bond formation, and interaction with catalytic residues critical for the modulation of DPP-IV. Findings from this study demonstrate the inhibitory potential and binding mechanism of CEO metabolites against DPP-IV for enhanced glucose homeostasis via glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide actions. Overall, CEOs afforded the identification of beta-terpineol as hit metabolite that can be studied as drug candidates towards type 2 diabetes care.

SL67 Hydrodistillation wastewaters from essential oils industry: a source of biologically active phyto-complexes with potential pharmaceutical and nutraceutical applications

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During the distillation process for essential oils production a variable, but significant, amount of wastewaters (DWWs) is produced, which are not yet addressed to any further application. DWW obtained from medicinal aromatic plants (MAPs) distillation can be likened to the product of a hot aqueous extraction, therefore rich in water-soluble metabolites with potential biological application once recovered as recommended by “European green deal” waste hierarchy, which prioritizes reuse and recycling. The scope of our progress work is to evaluate the phenolic composition of wastewaters coming from hydrodistillation of several MAPs belonging to the Labiatae family as well as to assess their potential biological activities for their exploitation in the pharmaceutical and nutraceutical fields. Our first results showed that DWWs contain high amounts of phenolic compounds [1, 2] such as organic acids, flavones and flavonols. Preliminary biological activity studies conducted highlighted the anti-inflammatory activity in intestinal epithelial cells [1] and on murine macrophage cells *in vitro* models and the inhibitory activity against α -Glucosidase and α -Amylase of these phytocomplexes [3]. Probiotic and antimycotic activity of oregano DWW has been also investigated. Our results suggest that growth and metabolic activity of probiotic *Lactobacillus spp.* were stimulated by oregano DWW. Also in presence of both *Lactobacilli spp.* and oregano DWW the growth of *Candida albicans* (an opportunistic pathogen) was significantly inhibited suggesting the potential use of DWWs in candidiasis prophylaxis and therapy. Although further studies are needed, our results open new possibilities for the use of MAPs DWW in the pharmaceutical, nutraceutical and food fields.

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SL68 Development of essential oil diffusion matrices using non-ionic surfactants-supported NADES and hydrophobic NADES

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Natural deep eutectic solvents (NADES) have emerged as a promising class of green solvents, offering an eco-friendly alternative to conventional organic solvents for extraction, reaction media, and product formulations [1]. In this study, we demonstrate that NADES, in combination with non-ionic surfactants, can form stable emulsions that effectively solubilize and release volatile organic compounds (VOCs) from lavender essential oil (LEO). Specifically, we investigated both hydrophilic and hydrophobic NADES to assess their performance in solubilizing and releasing 25 VOCs, identified via headspace analysis and gas chromatography-mass spectrometry (GC-MS). Multivariate analyses, including principal component analysis (PCA) and orthogonal partial least-squares discriminant analysis (OPLS-DA); revealed five distinct clusters (C, N1, N3, N5, and N9). While hydrophilic NADES excelled at initial solubilization, hydrophobic NADES provided a more controlled, sustained release, making them advantageous for long-lasting fragrance applications. These findings are consistent with additional research showing NADES can encapsulate lipophilic bioactive compounds in hydrogel matrices [2], while other carrier systems, such as starch matrices with enzymatically controlled degradation [3] and low-molecular-weight substances like succinic acid [4] have also demonstrated efficacy in modulating volatile release. Overall, this work underscores the critical role of the Hydrophilic-Lipophilic Balance (HLB) in NADES-based systems and shows how tuning surfactant concentrations can significantly influence VOC release. By highlighting both the challenges and opportunities for optimizing NADES compositions, this study contributes to the broader pursuit of sustainable solvent systems across cosmetics, pharmaceuticals, and fragrance industries. These insights foster new possibilities in sustainable fragrance technology.

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SL70 Ethnopharmacology in foods

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This study explores the ethnopharmacological dimensions of food through the lens of Symeon Seth's *Syntagma de alimentorum facultatibus*, an 11th-century Byzantine manual that examines the nutritional and medicinal properties of various foods [1]. While it may seem that late Byzantine medicine merely echoed the classical knowledge of Greek and Roman scholars and the Arab influence, Seth's manual reveals an innovative approach to food and health. By categorizing foods according to their effects on the body, both positive and negative, Seth introduces a nuanced understanding of how different food substances can affect human health. His work emphasizes the importance of balance, drawing from Hippocratic humoral theory, and provides a comprehensive guide to food as a therapeutic tool in maintaining health. By categorizing foods according to their physiological effects, Seth underscores the intricate relationship between diet and health.

Despite its historical significance, Seth's work has largely been overlooked in modern academic research, and this study aims to bridge that gap by exploring

its ethnopharmacological contribution by recording and comparing Seth's therapeutic claims with former historical and modern pharmacological data, including those from the European Medicines Agency. Through Seth's manual, the ethnopharmacology of foods is highlighting the influence of traditional dietary practices on health.

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SL71 Translating South African plant ethnobotanical reports into evidence-based data for neuroscience drug discovery: Low resource research environment screening tool

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South-African plants are rich in phytochemicals that could be a source of novel therapies for neurological disorders. The aim of this study was to validate a 5-step screening process in a resource-constrained research environment. The process was used to translate anecdotal and ethnobotanical reports into evidence-based data for lead discovery. The plant families of Apocynaceae (*Gomphocarpus fruticosus*), Lauraceae (*Ocotea bullata*), Melianthaceae (*Bersama lucens*), and Meliaceae (*Ekebergia capensis*) were selected from our database [1]. During phase-1, selected plant families (one plant/family) were subjected to a qualitative phytochemical and elemental analysis. Subsequently, chromatographic fingerprinting (Phase-2) and computational analysis (Phase-3) followed. Calcium (Ca) and potassium (K) were present in all plant families, while magnesium (Mg) and phosphorus (P) were undetectable in the lauraceae and meliaceae families, respectively. Flavonoids, phenolics, alkaloids, and terpenoids were present in all plant families. Furthermore, subsequent flavonoids (luteolin, quercetin, and rutin) and phenolics (6-Gingerol, chlorogenic acid and kaempferol) were confirmed by chemical fingerprinting in the apocynaceae and meliaceae families. The presence of macrominerals has been linked to better brain health. Additionally, phenolics, flavonoids, and alkaloids are known to have neuroprotective and neuromodulation potential. The results indicate the robustness of the screening process in generating preliminary data for the selection of the most promising plant family for the discovery of neuroscience drugs in low-resource research settings. Further studies are necessary using diseased neurological models *in vitro* (Phase-4) and *in vivo* (Phase-5) to complete the validation of the screening process and the comprehensive translation of ethnobotanical reports into evidence-based data.

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SL72 Traditional herbal medicinal products: evolving the use of adaptogens and immunomodulators

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The European Medicines Agency (EMA) recognizes several herbal medicinal products for indications such as “mental stress and mood disorders” and “fatigue and weakness,” which include well-known adaptogens (e.g., *Rhodiola rosea* L. rhizome and *Panax ginseng* C.A. Meyer root, respectively), as well as herbal medicines for “cough and cold”, which include immunomodulators (e.g., *Echinacea* spp., *Pelargonium sidoides* DC. root) [1]. These products have clear

therapeutic indications; however, their mechanisms of action remain complex and often non-specific, posing challenges for clinical interpretation and standard pharmacological modeling. This presentation aims to critically assess the evolution of scientific knowledge surrounding adaptogenic and immunomodulatory herbal medicinal products authorized in Europe. While traditionally used for stress resilience and immune support, these herbal medicines are now supported by a growing body of preclinical and clinical evidence. Recent studies have clarified the synergistic roles of individual phytochemicals within adaptogenic and immunomodulatory species [2–4]. At the same time, emerging tools such as artificial intelligence, bioinformatics, and microbiota research are enabling a more integrated and rational exploration of matrix pharmacology [5]. This presentation will outline how these innovations are reshaping both the scientific evidence and regulatory perspectives surrounding herbal medicinal products, setting the stage for a new era in their safe and effective use in clinical practice.

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SL73 The importance of the phytocomplex in the safety assessment of red yeast rice and plants containing hydroxyanthracenes

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The safety evaluation of botanical supplements requires a multidisciplinary approach that considers the phytocomplex as a whole, rather than focusing solely on isolated active constituents. This study, which reflects the approach of SIFITLab to botanicals under EFSA investigation, focuses on two representative cases: red yeast rice (RYR) and plants containing hydroxyanthracenes (HADs). For RYR, numerous samples with varying monacolin K (MK) content were analyzed via HPLC-DAD, revealing substantial variability not only in MK but also in secondary monacolins, triterpenes, and polyphenols. Enzymatic assays showed that the RYR phytocomplex exhibited superior HMG-CoA reductase inhibition compared to pure lovastatin (LOV), indicating possible synergistic effects. Moreover, *in vitro* testing on intestinal, hepatic, renal, and muscle cells demonstrated lower cytotoxicity of RYR compared to LOV. In muscle cells, qRT-PCR analyses confirmed a milder impact on the expression of genes related to damage, supporting a better safety profile [1]. In parallel, HAD-containing botanical extracts were compared to single HAD compounds in terms of stability, bioaccessibility, and intestinal toxicity. Simulated digestion and *in silico* modeling showed that HAD glycosides were stable but not absorbed, while aglycones exhibited limited bioaccessibility, highly dependent on the plant matrix. Importantly, whole extracts were far less cytotoxic than isolated HADs and did not affect oxidative or inflammatory markers in Caco-2 cells [2]. These results underline that the phytocomplex can significantly modulate both efficacy and safety. Future regulatory and scientific assessments should adopt phytocomplex-based evaluation strategies to ensure accurate and clinically relevant risk-benefit assessments of botanical preparations.

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SL74 Exploring the bioactivity of bilirubin and phyllobilins in atherosclerosis: functional insights and future mechanistic investigations

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Atherosclerosis-driven cardiovascular diseases remain the leading cause of mortality worldwide. Epidemiological studies suggest that individuals with slightly elevated serum bilirubin levels may have a lower risk of developing atherosclerosis [1]. Structurally related phyllobilins, derived from chlorophyll breakdown in plants, are found in dietary and medicinal plants, and may also influence health [2].

This study explores the potential roles of bilirubin and phyllobilins in cellular mechanisms relevant to atherosclerosis. To assess their bioactivity, we conducted a series of *in vitro* assays modeling processes involved in atherosclerosis. Using primary human cells—including human umbilical vein endothelial cells, human aortic smooth muscle cells, and peripheral blood mononuclear cells—we investigated the effects of five phyllobilins and bilirubin in atherosclerosis-relevant *in vitro* assays. Results demonstrated that bilirubin and phyllobilins were unable to inhibit the key event in the pathogenesis of atherosclerosis, the adhesion of leukocytes to inflamed endothelial cells. However, they exhibited intriguing bioactivities against other relevant processes: (i) They exhibited antioxidant activity by reducing oxidative stress *in vitro* and in living cells, which may help prevent lipid oxidation. (ii) Bilirubin but not phyllobilins significantly decreased oxidized low-density lipoprotein uptake by macrophages, a crucial step in foam cell formation. (iii) One phyllobilin, Cj-PxB, significantly suppressed vascular smooth muscle cell proliferation and migration. Both processes are central to plaque development.

While we describe several functional effects, the mechanism by which phyllobilins inhibit the functions of vascular smooth muscle cells and macrophages remain unknown. Further research is needed to determine potential therapeutic relevance in this context.

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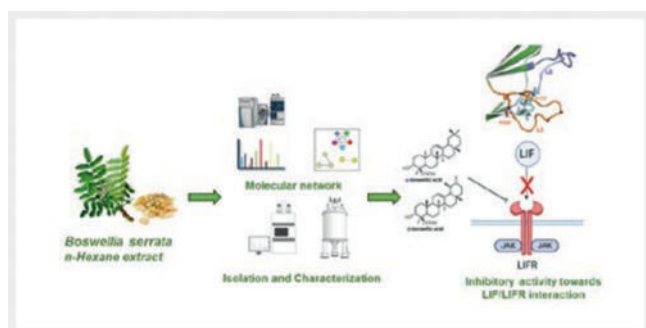
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SL75 *Boswellia serrata*: a rich source of triterpenes acting as antagonists of the leukemia inhibitory factor receptor

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Boswellia serrata, also known as Indian frankincense, is characterized by multiple beneficial effects, mainly correlated to anti-inflammatory activity [1–3]. In this study, we investigated its possible activity on targets never explored for *Boswellia*, mainly involved in hepatic fibrosis and cancer development [4]. The *n*-hexane extract exhibited a significant antagonistic activity on the interaction between Leukemia Inhibitory Factor (LIF) and its receptor (LIFR). A metabolomic-guided phytochemical investigation, based on a LC-HRMS/MS and Molecular Networking combined approach, was carried out on this extract. The following isolation and NMR analysis led to an unambiguous assignment of several *Boswellia* triterpenoid metabolites, including two new compounds, and a pharmacological evaluation to identify the bioactive *Boswellia* components responsible for the *n*-hexane extract activity on LIF/LIFR system. Key active metabolites, including boswellic acids and their derivatives and a small library of semisynthetic analogues, demonstrated potential inhibitory activity towards LIF/LIFR interaction. Among them, α -boswellic acid emerged as the best candidate LIFR antagonist, able to reduce the expression of col1 α 1 and α -SMA in LX-2 cells. Computational studies allowed to better understand the binding mode in the receptor, highlighting the role of the carboxyl group in engaging a network of electrostatic and hydrogen bond interactions within residues of hLIFR binding site. This finding suggests the potential use of *Boswellia* in hepatic fibrosis and sheds light on a relatively novel target for liver fibrosis therapy [5] (► Fig. 1).



► Fig. 1 Research workflow

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SL76 Osteoblast and osteoclast modulation by triterpenes and ellagitannins from *Bischofia javanica* leaves

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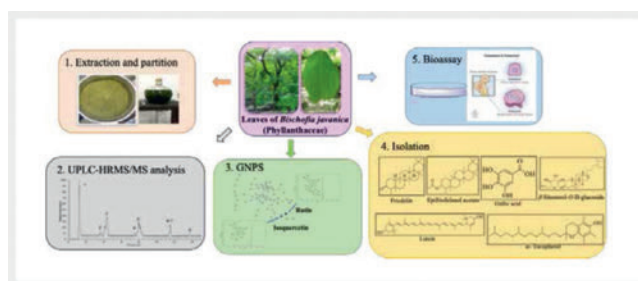
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Bischofia javanica ('茄冬 Jia Dong', Phyllanthaceae family), is traditionally used in Taiwan to treat joint inflammation, asthma, and infections. It is also consumed as a culinary ingredient to promote musculoskeletal strength, as reflected in folkloric practices by renowned artist Hsu Po-Yih (許伯夷).

Maintaining homeostasis requires balanced nutrition and immune regulation, both essential for muscle, organ, and nerve development, while imbalance contributes to chronic conditions like osteoporosis [1], diabetes, and cancer. Given its long-standing use and safety profile, *B. javanica* is a promising candidate for further biomedical investigation [2].

This study evaluated the effects of *B. javanica* extracts with different polarities on bone cell differentiation. The crude 80 % ethanolic (10 μ g/mL) and 80 % methanolic (80 μ g/mL) extracts significantly promoted osteoblast differentiation (induced ALP activity) and inhibited osteoclast formation (reduced TRAP activity). Notably, the hexane fraction (80 μ g/mL) suppressed both cell types, indicating dual regulatory activity. Methanolic fraction further inhibited neutrophilic inflammation and SARS-CoV-2 spike/ACE2 pseudovirus activity. UP-LC-MS/MS and GNPS molecular networking identified major compounds in the active methanolic fraction, including gallic acid, ellagic acid, geraniin, corilagin, and vitexin. Additional compounds isolated from the *n*-hexane fraction included major gallic acid, vitamin α -tocopherol, carotenoid lutein and triterpenes friedelin and epifriedelanol acetate (► Fig. 1). Structural characterization was confirmed via 1D/2D NMR and mass spectrometry. Key constituents – gallic acid, geraniin, and friedelin – demonstrated dual action by promoting osteoblast formation and/or inhibiting osteoclastogenesis.



► Fig. 1 Overview of the *Bischofia javanica* study

These findings support the potential of *B. javanica* as a therapeutic agent for bone-related disorders, particularly osteoporosis.

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SL77 Interactions in medicinal plant-based antimicrobials: synergy, antagonism and all that goes in between

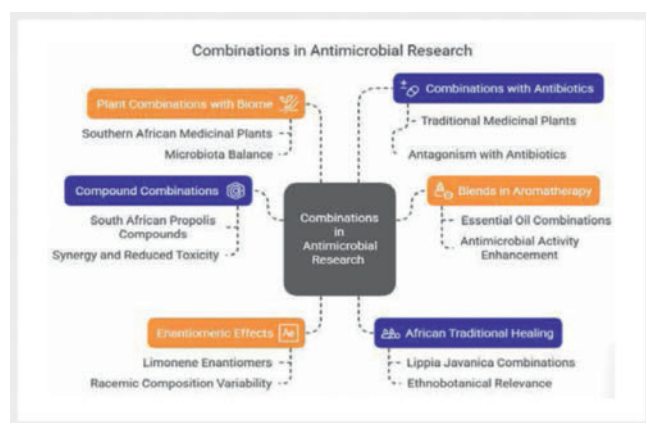
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The antimicrobial properties of medicinal plants are not only influenced by their individual activities but also by the intricate interactions. These interactions, including synergy, antagonism, and what goes in between are pivotal in understanding antimicrobial efficacy in combined blends.

This study aims to explore the various types of antimicrobial interactions providing examples from our studies focusing on several interaction types: (1) Compound combinations between South African propolis compounds flavonoids pinocembrin, galangin, and chrysin which demonstrate synergy, while exhibiting reduced toxicity in combination (2) Blends used in aromatherapy, where more than 1500 essential oil combinations were analysed and an intricate analysis of synergistic, antagonistic, additive and non-interactive interactions are reported. Results demonstrate the value of using carrier oils for an enhanced antimicrobial effect, as well as reducing toxicity (3) Enantiomeric effects, using the model compound limonene where the combination with other essential oil compounds demonstrate variable interactions depending on the racemate studied (4) African traditional healing using the example of *Lippia javanica* in combination with other medicinal plants against respiratory pathogens (5) Plant combinations with the biome where plants used to treat gastric ulcers have been explored and their interactions with the gut microbiome demonstrate selective synergy, and lastly, (6) Combinations with conventional antibiotics where we explored the effects of traditionally used medicinal plants collected from *Muthi* markets with antibiotics. The prevalent antagonism found was alarming (► Fig. 1).



► Fig. 1 Oral presentation plan

This research highlights the need for a multidimensional approach to studying medicinal plant combinations. It opens new avenues for developing novel, effective, and sustainable antimicrobial therapies, particularly in the context of global antimicrobial resistance.

SL78 Discover and development of quorum sensing inhibitors for modulation of plant-microbe interactions

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Quorum sensing (QS) is a bacterial communication mechanism based on the production and detection of signal molecules such as *N*-acyl homoserine lactones (AHLs), which regulate collective behaviors including biofilm formation, virulence, and symbiosis [1, 2]. The disruption of this system, referred to as quorum quenching (QQ), constitutes a promising strategy for controlling pathogenic microorganisms and promoting beneficial plant-microbe interactions [2, 3]. In this work, we present an integrative strategy for the identification and development of natural QS inhibitors (QSI) using a combination of chromatographic techniques, bioautography, molecular docking, and *in vitro* microbiological assays [1, 4]. Previous findings from our group identified the piperamides piperine and trichostachine, isolated from *Piper nigrum*, as active QSIs that reduce violacein production in *Chromobacterium violaceum* CV026 without affecting bacterial growth. Docking analyses confirmed their affinity for the CviR receptor [4]. Building upon this approach, we report novel and unpublished data on *Asterohyptis* sp., a native Mexican Lamiaceae plant. Phytochemical analysis of its ethanolic extract revealed the presence of caffeic acid esters with high *in silico* binding scores against CviR and LasR receptors. Preliminary *in vitro* assays demonstrated that these compounds inhibit violacein synthesis and biofilm formation in *C. violaceum* and *Pseudomonas aeruginosa*, respectively. These findings highlight the potential of acylated phenolic esters as natural QSIs capable of modulating QS-dependent bacterial behavior. This study emphasizes the value of endemic plant species in the discovery of novel bioactive compounds and contributes to the development of sustainable strategies for managing plant-associated microbial communities in agriculture.

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SL79 Inhibition of human topoisomerases I and II and protein tyrosine phosphatase 1B by longchain compounds from lichens

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Lichens produce a diverse array of metabolites, many of which have promising bioactivities but yet remain underexplored. Among others, metabolites comprising side chains of increased length seem to be associated with noticeable bioactivities [1].

Within this study, metabolite profiling of five lichen species was conducted and seven long-chain lichen-derived metabolites – five depsides (glomellic acid, glomelliferic acid, divaricatic acid, stenosporic acid, and perlatic acid), one depsidone (α -collatolic acid), and one diphenylether (β -collatolic acid) – were isolated using chromatographic methods. Aiming to identify new inhibitor scaffolds and to understand the impact of the length and functionalization of the side chains, they were investigated on their potential to inhibit human DNA topoisomerases I and II (hTopo I and II), therapeutic targets of anticancer agents, and protein tyrosine phosphatase 1B (PTP1B), a target whose inhibition is expected to be beneficial in the treatment of diabetes mellitus, in cell-free assays. The results demonstrated that, except for glomellic acid, all tested compounds effectively inhibited hTopo I and II. Similarly, these metabolites displayed significant inhibitory effects against PTP1B, with IC₅₀ values ranging from 4.01 to 19.7 μ M. Molecular docking studies confirmed these results.

These findings highlight the considerable potential of long-chain lichen metabolites and underline that the structural diversity of lichen compounds represents a valuable resource for developing novel bioactive molecules.

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SL80 Quality and safety considerations for traditional kratom and commercial products labelled as kratom

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Kratom is the common name attributed to *Mitragyna speciosa* Korth., a deciduous tree of the Rubiaceae family native to Southeast Asia. Historically kratom leaves were chewed or consumed as a tea to mitigate fatigue and alleviate pain, particularly among manual laborers [1]. The alkaloids are recognized as the primary active compounds representing 0.5–3% of the total dry weight, with mitragynine reported to comprise of 51–82% of the alkaloid composition [2, 3]. Other pharmacologically active alkaloids found in leaf include paynantheine, speciogynine, speciociliatine, and mitraciliatine [1, 3]. With growing demand over the last decade, kratom leaves are primarily harvested from cultivated trees which has had an impact on the alkaloid distribution and consequently the safety profile [3]. Another alkaloid, initially thought to be present in native kratom leaf, 7-hydroxymitragynine (7-OH) is now recognized as a human metabolite of mitragynine and a post-harvest oxidative by-product found in trace levels ranging from 0.03–0.40 mg/g dry leaf [2–4]. Recently, there have been

a plethora of products comprised of predominately 7-OH flooding the market, many of which are erroneously labelled as “kratom extracts”. Given that 7-OH is 3–22 times more potent than morphine at opioid receptors and demonstrates abuse liability, these products pose a public health risk [2, 5]. This study aimed to evaluate the phytochemical implications of the changing supply chain from wild-crafted to cultivated leaf and review commercial products readily available in the U.S. market in consideration of regulatory and safety implications for public health.

Disclosures: PB serves as a scientific expert and regulatory consultant on botanical dietary ingredients, including kratom. No clients had any contribution or input into this study. MC has nothing to disclose.

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SL81 Advancing botanical safety through collaborative innovation

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Botanical ingredients — derived from plants, algae, and fungi — are widely used in foods, supplements, cosmetics, and herbal medicinal products. Despite their popularity, evaluating the safety of these complex mixtures poses a significant challenge. Traditional toxicity testing methods, developed for single, well-defined chemical entities, are often ill-suited to capture the multifaceted nature of botanicals, which can contain hundreds of constituents that vary by source, preparation, and use. As a result, there is an urgent need for innovative approaches that can more accurately assess potential risks while supporting the continued development and safe use of botanical products. To meet this need, ongoing efforts have focused on developing and applying New Approach Methodologies (NAMs) to evaluate key toxicity endpoints relevant to botanical safety, such as liver toxicity, genotoxicity, and developmental and reproductive toxicity (DART), among others. Progress includes establishing systematic frameworks for literature review, authenticating botanical test materials, and integrating advanced chemical analyses and ADME modeling to improve interpretation of in vitro data. Select case studies using botanicals known for safety concerns, like aristolochia and kava, have helped stress-test these methods and refine assay approaches. Looking ahead, the focus is on expanding the relevance and predictive power of these tools across a broader range of botanicals and real-world exposure scenarios. This includes improved integration of in vitro and in silico data, closing key gaps in predictive modeling, and ensuring that

scientific advances translate into regulatory practice. Training, transparent data sharing, and global engagement remain central to these efforts.

SL82 Quality of food supplements – a never ending story of food fraud in our real world

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Herbal food supplements (syn. botanicals, [1]) are widely consumed for their potential health benefits. However, quality of these products can vary due to differences in raw material sources, processing methods, and potential adulteration. Ensuring authenticity, purity and consistency of these products is essential for consumer safety and product efficacy. This study focuses on the development and validation of analytical methods for quality assessment of herbal food supplements. Analytical techniques were developed for extracts from *Passiflora incarnata*, *Vaccinium macrocarpon*, *Silybum marianum*, and *Boswellia serrata*. For passionflower, a UPLC method was validated to quantify flavone C-glycosides. A clear difference between registered drugs and food supplements was visible. Only one food supplement showed positive identity, while all others did not meet specifications. In contrast, all registered passion flower herbal drugs were of high quality. Similar, cranberry (*Vaccinium macrocarpon*) food supplements were analyzed using HPLC for anthocyan-fingerprinting and photometric methods to determine proanthocyanidin levels. Again, food fraud was obvious for some preparations. The data presented here clearly reveal again massive deficits in quality of food supplements, and intensified quality control and quality surveillance by authorities by use of unambiguous and validated methods must be demanded.

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SL83 Ashwagandha: Evaluating its safety for human consumption

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Ashwagandha (*Withania somnifera* (L.) Dunal, Solanaceae) is a traditional herb used in Ayurvedic medicine. It is regarded as an adaptogen because it enhances an organism's ability to tolerate, adapt to, and survive stress. The use of adaptogenic ingredients in dietary supplements and herbal medicines has grown in popularity. Among adaptogens, ashwagandha has shown the most rapid growth in the herbal supplement market. Evaluations of ashwagandha's regulatory status in the EU have classified it as a food supplement. While it has traditionally been considered safe based on prolonged use, EU regulators have expressed concerns about the safety of ashwagandha as a supplement ingredient since 2013, culminating in a recent recommendation for an Article 8 procedure according to Regulation (EC) No 1925/2006 by the Heads of Food Safety Agencies working group "Food Supplements." This process could lead to a ban on ashwagandha as a food supplement ingredient and, consequently, the effective removal of the ingredient from the EU marketplace altogether. Primary concerns regarding the safety profile of ashwagandha include its effects on the female reproductive system, male reproduction due to its impact on sex hormone levels, thyroid hormones, inhibition of acetylcholinesterase, the immune system, and hepatotoxicity (based on case reports). These concerns raise questions about safe dietary exposure and effects on vulnerable groups,

including older adults and pregnant or breastfeeding women. Additionally, there is interest in whether ashwagandha preparations from root, leaf, and mixed plant parts have different safety profiles. This presentation summarizes findings compiled in a special issue of *Phytotherapy Research*, reviewing over 100 clinical trials for safety-related outcomes.

SL84 Monographs and scientific evidence in the era of AI – unde venis – quo vadis ESCOP?

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The landscape of scientific evidence generation and dissemination, as well as the demand for and retraction of knowledge by audiences, is undergoing a major shift with the emergence of artificial intelligence (AI). However, the knowledge base on the traditional use of herbs—and the datasets on which AI systems are trained—remains largely intransparent to users. Monographs, particularly within institutions like the European Scientific Cooperative on Phytotherapy (ESCOP), are still developed through careful review of primary literature, typically applying PRISMA or similar criteria to assess study quality. Based on its 35-year history (unde venis) [1], ESCOP's approach in parallel of more regulatory-driven monographs from public European institutions—has long served as a key reference for the traditional and well-established use of herbal medicines.

Yet in an era of data abundance and algorithm-driven synthesis, the question arises: quo vadis? AI enables real-time literature analysis, dynamic meta-analyses, and machine-assisted guideline development—putting traditional static monograph systems at risk of obsolescence unless modernized. Initiatives like Herbal CONSORT [2], which promotes standardized reporting in herbal trials, and Empowered by Evidence [3], a platform for reliable herbal research, are shaping a new evidence culture that fits AI's potential. The founding of the WHO Traditional Herbal Medicine Centre further signals a global shift toward uniting traditional knowledge with scientific rigor.

ESCOP is now part of the Global Alliance of Integrative Health and Medicine (GAIHM), fostering international collaboration between researchers and practitioners. To stay relevant, ESCOP must adopt AI-supported workflows, collaborate with these emerging networks, and evolve its monographs into living, adaptable tools reflecting ongoing scientific and clinical developments.

Natural language processing and science-based algorithms should be applied thoughtfully and competently—rather than relying solely on mainstream prompts or general-purpose chatbots [4].

This convergence of tradition and innovation presents a unique opportunity to reinforce the scientific foundation of herbal medicine in the 21st century.

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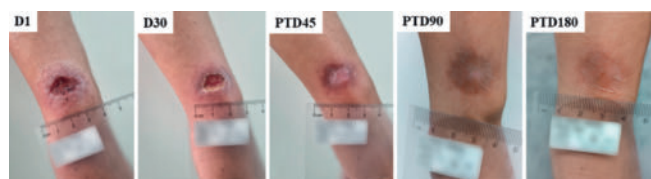
SL85 Topical Arnica tincture as a promising therapeutic alternative for cutaneous Leishmaniasis: Evidence from a phase Ib/II clinical trial

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Cutaneous leishmaniasis (CL) is caused by various species of *Leishmania* parasites. It has a global incidence of 1.2 million cases annually, primarily in the Americas and the Eastern Mediterranean region. Despite the continued use of pentavalent antimonials as the standard treatment, their toxicity has prompted the search for alternative therapies [1]. *Arnica montana* L. (Asteraceae) is widely recognized as a phytotherapeutic plant due to its anti-bruising, analgesic and antimicrobial properties. The tincture obtained from the flowers has demonstrated excellent *in vivo* activity against CL in a hamster model [2] and high skin permeability and retention in the epidermis without systemic circulation, making it a promising option for topical treatments [3]. A phase Ib/II clinical study (NCT05094908) was conducted to evaluate the safety, tolerance, and therapeutic response of arnica tincture in patients with CL [4]. Sixteen participants aged 18 to 65 years, all diagnosed with CL, were randomly assigned to receive Arnica tincture, applied topically three times daily for either 30 or 45 days. Of the 12 participants who completed the 180-day follow-up, all achieved complete lesion healing, demonstrating a 100% cure rate (► Fig. 1). The remaining four participants withdrew their informed consent before or during treatment. The most common adverse events were mild and included erythema, pain, edema, and a burning sensation at the application site. No severe adverse effects were reported.



► **Fig. 1** Appearance of CL of a representative participant treated with arnica tincture for 30 days. TD0: Before treatment; D30: last day of treatment; PTD45: day 45 after end of treatment; PTD90: day 90 after end of treatment and PTD180: day 180 after end of treatment. Arnica tincture demonstrated promising results in treating CL and exhibited a favorable safety profile. However, due to the small sample size and absence of comparison with conventional therapies, a further phase III study with more robust methodological design has been initiated to assess its efficacy accurately.

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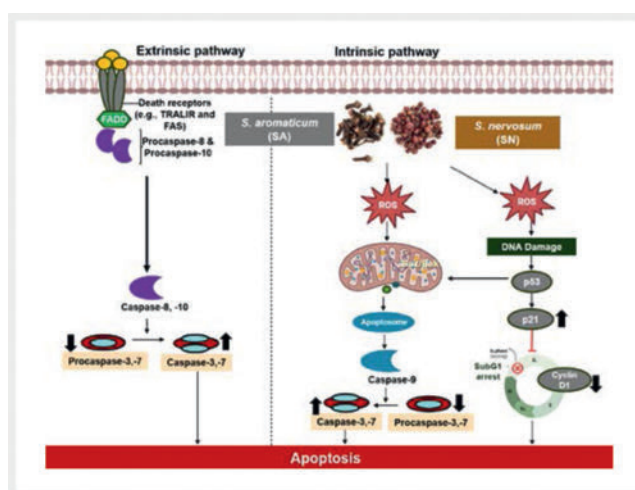
SL86 Phytochemical analysis and anticancer activity of *Syzygium* spp. extracts against colorectal cancer cells via apoptosis and cell cycle arrest

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Colorectal cancer (CRC) is the third most common cancer globally, accounting for a significant proportion of cancer-related morbidity and mortality worldwide. This study investigated the antiproliferative and pro-apoptotic effects of *Syzygium aromaticum* (SA) flower buds residue from hydrodistillation extract and *Syzygium nervosum* (SN) seeds extract in CRC cell lines HCT116 (p53 wild-type) and HT-29 (p53 mutant). SA and SN extracts were subjected to phytochemical profiling using LC/MS/MS. Major constituents identified or tentatively identified in SA were gallic acid, strictinin, biflorins, and ellagic acid, while SN contained gallic acid, pedunculagins, ellagic acid, and 2',4'-dihydroxy-6'-methoxy-3',5'-dimethylchalcone (DMC). Quantitative analysis revealed higher levels of gallic acid (2.68%) and ellagic acid (6.70%) in SA compared to SN (0.26% and 3.06%, respectively). Both extracts demonstrated strong DPPH free radical scavenging activity (SA: IC₅₀ 8.75 µg/mL, SN: 12.92 µg/mL) and selectively inhibited proliferation of HCT116 cells by over 50%, with less effects in HT-29 cells. Apoptosis induction was confirmed in HCT116 via Annexin V-FITC/PI staining, Caspase-3/7 activation, and elevated Caspase-3 expression. Cell cycle analysis revealed sub-G1 arrest with upregulation of p21 and downregulation of Cyclin D1. No significant molecular mechanism change was observed in HT-29 cells. These findings suggested that SA and SN extracts, rich in phenolic constituents, possessed selective anticancer activity in p53-competent CRC cells, and could be promoted as phytotherapeutic agents for CRC treatment (► Fig. 1).



► **Fig 1** Anticancer Activity of *Syzygium* spp. Extracts Against Colorectal Cancer Cells via Apoptosis and Cell Cycle Arrest

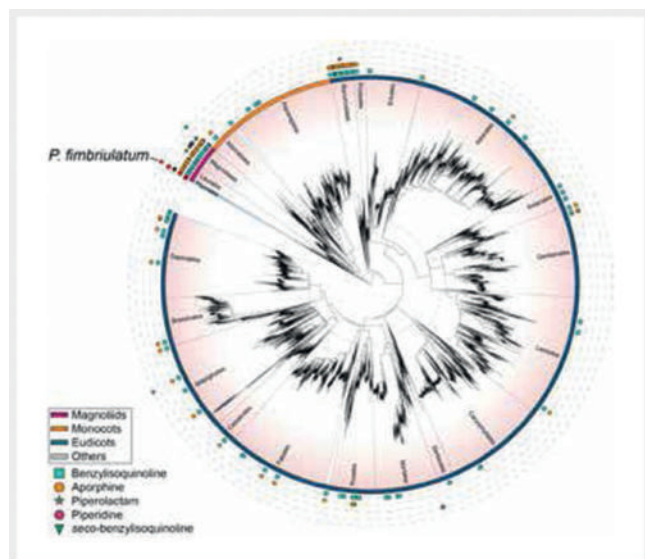
SL87 Computational metabolomics reveals overlooked chemodiversity of alkaloid scaffolds in the *Piper* genus

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Plant specialized metabolites play key roles in physiological processes and ecological interactions. Identifying novel metabolites, as well as discovering known compounds in new species, is crucial for answering broader biological questions. The *Piper* genus (Piperaceae family) is known for its special alkaloids biochemistry, with more than 300 alkaloids (“piperamides”) reported to date. In a recent study [1], we investigated the alkaloid diversity of *Piper fimbriatum*, a myrmecophytic plant native to Central America, using a metabolomics workflow that combines untargeted LC-MS analysis with a range of state-of-the-art computational tools. Specifically, we leveraged open MS/MS spectral libraries and metabolomics data repositories for metabolite annotation and guided isolation efforts toward structurally-novel compounds. As a result, we identified 22 alkaloids belonging to 5 different classes, and isolated one novel seco-benzylisoquinoline alkaloid carrying a linear quaternary amine moiety which we named *fimbriatumine*. Notably, several of these alkaloids had never been reported in *Piper* plants, despite their extensive phytochemical investigation. To support this discovery, we sampled 12 additional *Piper* species and confirmed the presence of these alkaloids across the genus. Finally, we contextualized our findings within a broader biological framework by systematically mining literature reports of the identified alkaloid scaffolds across angiosperms. Specifically, we used the Wikidata knowledge base [2] for automated literature mining and mapped the results onto the angiosperm tree of life [3] (► Fig. 1). Our findings expand the known Piperaceae alkaloid diversity and demonstrate the value of revisiting well-studied plant families using modern computational metabolomics.



► Fig. 1 Angiosperm tree of life [3] mapped with literature reports, mined from Wikidata [2], of the alkaloid scaffolds discovered in the study [1].

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SL89 Eudragit-coated liposomal delivery system of *Salvia macilenta* Boiss.: phytochemical profiling and anti-inflammatory activity

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Salvia genus belonging to the Lamiaceae family, is known for its pharmaceutical activities, including analgesic, anti-inflammatory, and antibacterial properties [1]. Among its species, 60 are grown in Iran and they have been utilized in folk medicine for disease prevention and treatment worldwide. In this context, *Salvia macilenta* Boiss, an aromatic subshrub endemic to southern Iran, has not been extensively studied, except for essential oil analyses [2, 3]. Therefore, the aim of the study was to define the phytochemical profile of *S. macilenta*, enhance the stability of the extract through nanoformulations, and evaluate its anti-inflammatory activity. The phytochemical investigation of *S. macilenta*'s aerial parts was conducted using high-resolution liquid chromatography coupled with mass spectrometry. This led to the identification of a new rearranged nor-abietane diterpenoid and 31 known metabolites, including flavonoids, phenolic compounds, and terpenes. Structural elucidation was performed through 1D and 2D NMR, HRMS, and comparisons with literature. To improve the stability and bioavailability of the extract, *S. macilenta* was incorporated into Eudragit-coated liposomes. The nanoformulation exhibited favorable physicochemical properties, excellent storage stability over three months, and high entrapment efficiency for rosmarinic acid (83.24%) and carnosol (51.19%). *In vitro* studies on LPS-stimulated HepG2 cells, used as model, demonstrated that the liposomal formulation significantly enhanced the extract's protective effects against oxidative stress and inflammation. This was evidenced by the upregulation of antioxidant proteins and a reduction in pro-inflammatory markers. These results suggest that the developed nanoformulation holds promise as a delivery system to enhance the therapeutic potential of *S. macilenta* extract.

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SL90 Perspectives on naturally occurring saponins as immunomodulatory and cytotoxic agents from Turkish plants

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Saponins are a diverse group of naturally occurring plant secondary metabolites present in a wide range of materials ranging from legumes and green leaves to sea creatures. They exhibit numerous biological activities such as fungicidal, antimicrobial, antiviral, anti-inflammatory, anticancer, antioxidant and immunomodulatory effects [1–3]. Considering the great potential of saponins, the main goal is placed on the isolation, purification and biological activities of saponins from Turkish plants. Saponins with adjuvant potential were determined as a result of *in vitro* immunomodulatory, cytotoxic and hemolytic activity studies performed on 19 different saponins from eight *Cephalaria* species. The structures of them were determined by advanced spectroscopic techniques such as 1D, 2D NMR and HR-MS. Immunomodulatory activity studies were conducted using QS-21 saponin in whole blood cells stimulated with PMA plus ionomycin as a positive control. Cytotoxicity studies were performed on HeLa, A549 and normal cell line HEK293 using the MTT method, and hemolytic activity studies were performed on human erythrocytes. It has been determined that saponin called elmalienoside A obtained from *C. elmalienensis* increase IL1- β production and didn't show any cytotoxic effect on cells and choose an adjuvant candidate for vaccine formulations. One of the imperative results obtained of this study was that the most important isolated component, holothurin saponins, found in sea cucumber *Holothuria tubulosa* were exhibited potent cytotoxic and apoptosis-inducing effects on PC-3 and PANC-1 cells highlighting its potential against prostate and pancreatic cancers. Considering these results, the isolated saponins have great potential for planning further studies for use in cancer therapy.

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Posters

P1 *In silico* bioprospection of phenolics against penicillin binding protein (PBP1A) of *Acinetobacter baumannii* for interventive therapy

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Infections caused by carbapenem-resistant *Acinetobacter baumannii* can result in death within 48 hours of hospitalization, with an overall mortality rate of about 68 %. The decreased affinity of β -lactams for Penicillin-Binding Proteins 1A (PBP1A) of *A. baumannii* has been identified as one of its resistance mechanisms. This study utilized a structure-activity relationship pharmacophore approach complemented with molecular docking to screen a library of 12000 phenolics with affinity for PBP1A of *A. baumannii*. Thereafter, the top five phenolics with the most negative docking scores and efficient drug-like features relative to the reference antibiotic (imipenem) were thermodynamically evaluated through a 300-ns molecular dynamics (MD) simulation. Interestingly, the top five leads (ZINC12659587 (–25.20 kcal/mol), ZINC59466627 (–35.01 kcal/mol), ZINC04989868 (–19.67 kcal/mol), ZINC71285349 (–17.90 kcal/mol), and ZINC58836805 (–11.95 kcal/mol) had better affinity for PBP1A than imipenem (–11.50 kcal/mol) with ZINC59466627 being the most potent of the

phenolics. A further probe into the structural architecture of the resulting complexes revealed that PBP1A became more compact and stable following binding the top five phenolics, with reduced amino acid residue fluctuations at its active site. In addition, except for ZINC12659587, quantum calculations using the DFT B3LYP/6-31G + (dp) revealed that the top five leads are more reactive than imipenem. While the findings from this study lent scientific credence to the profiled phenolics as modulators of PBP1A of *A. baumannii*, further studies using *in vitro* and *in vivo* models are underway to validate the potency of the compounds in the management of infections caused by *A. baumannii* [1–3].

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P2 *In vivo* anti-diabetic agent from the “insulin plant” *Costus igneus*

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Costus igneus Nak, belonging to the Costaceae family, is a perennial plant native to South and Central America, commonly known as “insulin plant” due to the use of its leaves in the traditional medicine for the treatment of diabetes [1]. Diabetes is a chronic metabolic disorder with a prevalence increasing worldwide, motivating the investigation of new active agents among natural sources with less side effects compared to synthetic drugs [2]. Several studies demonstrated the potential of *C. igneus* leaves in the treatment of diabetes [3,4], but further studies are needed to elucidate the hypoglycaemic action of the plant and its components. The aim of this study was to isolate anti-diabetic compounds from *C. igneus* rhizome collected in Uyo (Nigeria). Dried and powdered rhizome was extracted with ethanol 99 %, then dissolved in water and partitioned with dichloromethane, ethyl acetate, and n-butanol. All the extracts were assayed in alloxan-induced diabetic rats (dose 1000 mg/kg) showing significant reductions in blood glucose level being dichloromethane extract the most active during both the acute and prolonged studies. The dichloromethane extract was then subjected to chromatography to obtain five sub-fractions with fraction 4 showing the highest anti-diabetic activity. The fractionation of fraction 4 led to the isolation of a pure diosgenin triglycoside, identified by 1D and 2D NMR, and ESI-MS as aferoside A. Aferoside A at dose of 100 mg/kg showed significant reduction of blood glucose level (51.9 % vs 64.8 % of glibenclamide at 10 mg/kg), highlighting its potential in the use of management of diabetes.

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P3 Traditional medicine in transition in Uganda: Past, present and future

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Traditional medicine has played a significant role in Uganda's healthcare system, with traditional birth attendants, bone setters, and herbalists being key practitioners. Despite advancements in modern medicine, a considerable portion of the population continues to rely on traditional medicine due to accessibility, cultural beliefs, and affordability [1]. This study explored the role of traditional medicine in Uganda, and how it has been evolving over time. We assessed the changes in practices, perceptions, and beliefs of traditional medicine and how it is integrated with conventional medicine in Uganda. The study employed ethnobotanical techniques, including interviews and focus group discussions [2] with practitioners and healthcare professionals, as well as a review of policy documents. We interviewed over 120 practitioners throughout Uganda, representing all regions and a range of practices in the country. The results indicate a gradual shift in the perception of traditional medicine, with increasing efforts to regulate and formalize its practice. While traditional birth attendants have faced restrictions due to maternal health policies, traditional bone setters and herbalists continue to operate, often integrating modern medical knowledge into their practice. The study also highlighted the challenges of regulating traditional medicine, including inadequate documentation, lack of standardized training, and tensions with formal healthcare providers. The research concludes that despite these challenges, traditional medicine remains a crucial aspect of Uganda's healthcare system. Efforts to harmonize traditional and modern practices could enhance patient outcomes and preserve valuable indigenous knowledge. Integrating traditional medicine into Uganda's formal healthcare system, supported by research and policy development, could improve healthcare accessibility and foster collaboration between traditional and modern practitioners.

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P6 Fish disease pathogens: Antibacterial, anti-biofilm and anti-quorum sensing activities of selected medicinal plant extracts

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Fish diseases such as kidney disease, dropsy and vibriosis pose serious challenges to the aquaculture industry, causing significant mortality. Antibacterial, anti-biofilm and anti-quorum sensing properties of selected plant extracts against reference strains and prevalent bacterial pathogens isolated from fish-

es were investigated. Acetone, ethanol and aqueous leaf extracts of *Alchornea laxiflora* (AL), *Ficus exasperata* (FE), *Morinda lucida* (ML), *Jatropha gossypifolia* (JG), *Ocimum gratissimum* (OG) and *Acalypha wilkesiana* (AW) were screened against fish bacteria (five reference strains: *Citrobacter freundii* ATCC 8090; *Aeromonas hydrophila* ATCC 35654; *Edwardsiella tarda* ATCC 15947; *Pseudomonas fluorescens* ATCC 135225; *Vibrio parahaemolyticus* ATCC 17802; *Vibrio alginolyticus* ATCC 17749; and four clinical isolates: *Lactococcus garviae* B 970/23; *Photobacterium acumselae* B44/16; *Vibrio alginolyticus* B789/2000). Acetone extracts of ML, JG and AW, the ethanol extract of AW, and aqueous extracts of AW and JG all had strong antibacterial activity, each showing excellent minimum inhibitory concentrations (MIC < 0.1 mg/ml) against tested organisms. The reference strains were more resistant than the clinical isolates. The extracts were screened against Vero and Caco-2 cells with tolerable safety margins. The best anti-biofilm (ABF) activity was displayed by ethanolic extracts of OG and AW, aqueous ML extract and ML and FE acetone extracts with ABF > 50% after 24 hours. Most plant extracts had selective minimum quorum sensing inhibition concentrations (MQSICs) against *Chromobacterium violaceum*. These findings highlight a promising alternative to conventional anti-infectives in the form of plant feed additives with potential use in controlling fish pathogens, particularly in terms of their efficacy against biofilm formation.

P7 Metabolomic landscape of *Helianthus annuus* hybrid seeds feeds the cheminformatic discovery of *Klebsiella pneumoniae* MrkH quorum sensing inhibitors

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Sunflower (*Helianthus annuus*) seeds are underexplored, yet phytochemical-rich, with many potential health benefits. *H. annuus*-based metabolites could also be channeled toward the search for novel quorum sensing inhibitors (QSIs), a new class of antimicrobials. Hence, this study modelled sunflower hybrid seeds metabolites against the key QS system protein (MrkH) of *Klebsiella pneumoniae*, a critical priority pathogen requiring novel therapeutics. Following the metabolomic profiling of hybrid seed extracts, *H. annuus* metabolites were modelled against the MrkH protein using advanced computational methods. Molecular docking revealed best binding poses for 5 lead metabolites (calceolarioside A, dicaffeoyl quinic acid, guanosine, cynarin, xanthosine) with a docking score range of -8.8 to -7.8 kcal/mol, relative to the autoinducer (-5.8 kcal/mol). Pharmacokinetics revealed that guanosine and xanthosine are explorable within the Lipinski rule of 5 (LRo5), and other leads beyond the Ro5. Dynamics simulation showed that all leads had higher negative binding free energy (ΔG_{bind}) in the range -42.54 kcal/mol (dicaffeoyl quinic acid) to -28.67 kcal/mol (guanosine) compared to the autoinducer (-10.85 kcal/mol) and cinnamaldehyde standard (-15.88 kcal/mol). All leads had stable interactions with MrkH active site residues (Arg106, Arg107, Asp109, Arg111, Asn183, Gln201, Ser140). Lead ligands-MrkH complexes were thermodynamically stable and compact, with an increased number of hydrophobic and hydrogen bond interactions. The lead metabolites from *H. annuus* hybrid seeds could be explored as new MrkH QS system inhibitors after conducting further in vivo and in vitro validation steps. Sunflower seeds thus hold significant potentials for drug discovery and therapeutics development, among other possible industry applications.

P8 Cytotoxic, anti-inflammatory, antioxidant effects of *Cassia abbreviata*, a medicinal plant used in traditional medicine in South Africa

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Medicinal plants have been used as remedies to treat diseases afflicting humanity. Among many diseases treated with plant-based remedies in South Africa include various cancers. The use of traditional medicine has been, in recent years, overshadowed by profound investment in promotion of Western medicine, based mainly on a single compendial active ingredient. Herbal medicine, on the other hand, relies on the combined activities of more than one compound, contributed by more than one species, in herbal preparations. The involvement of more than one active compound in herbal concoctions may be effective, as targeted drug resistance may be difficult against a collection of phytochemicals in a mixture. Therefore, this study aimed to demonstrate the anticancer, antioxidant and anti-inflammatory activities of *Cassia abbreviata*. Plant leaves were extracted with acetone and tested for their cytotoxic effects against various cancers using the MTT assay, for antioxidant activity using radical scavenging method, anti-inflammatory activity, as well as antimetastatic activity using the scratch assay. Moderate antioxidant activity was observed at all tested concentrations. Cell viability reduced to $\pm 60\%$ at 0.5 mg/ml, while displaying a dose-dependent effect on proliferation of MCF7 cells. The extract had an antimetastatic effect, inhibiting growth of cells in the wound area. The plant extract reduced ROS activity in LPS-induced RAW 264.7 cells and NO production. The findings of this study strengthen claims of anticancer activities of the plant species. Further studies are underway to ascertain mode of cell death and the pathways through which the plant exerts its biological activities.

P9 Valorization of orange peel waste for bioactive nanocomposites: green extraction of flavonoids coupled with carbon quantum dots for enhanced antibacterial activity and wound healing

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Orange peel waste, a byproduct of the citrus industry, is a rich source of bioactive compounds like flavonoids which are known for their potent antioxidant, antimicrobial, and anti-inflammatory properties. This study focuses on the sustainable extraction of flavonoids from orange peel waste using natural deep eutectic solvents (NADES) and their application in wound healing. The NADES system, optimized at a 1:1 molar ratio of choline chloride and lactic acid, demonstrated high efficiency, yielding stable and bioactive flavonoid-rich extracts. To further enhance the therapeutic potential of these extracts, carbon quantum dots (CQDs) were employed as nanocarriers. CQDs, renowned for their biocompatibility and unique photoluminescence properties, improved

the stability and antimicrobial efficacy of the flavonoids. The conjugates were characterized using spectroscopic techniques, revealing an increased fluorescence intensity from 7500 (CQDs alone) to 9500 (CQDs-flavonoid conjugate) at an emission wavelength of 433 nm, confirming successful conjugation. The antimicrobial efficacy of the CQDs-flavonoid conjugates was assessed against wound-associated pathogens, including *Escherichia coli*. The conjugates demonstrated a 16 mm zone of inhibition against *E. coli* at a concentration of 100 µg/mL, showing moderate antimicrobial activity compared to the positive control (standard drug, 28 mm). Additionally, in vitro wound-healing assays with L929 fibroblast cells revealed that the conjugates, at a concentration of 500 µg/mL, achieved 45.63 % wound closure within 48 hr, significantly outperforming the control (20.36 %), but remaining lower than the standard (85.63 %). These findings lay a strong foundation for further exploration of CQDs-flavonoid conjugates in antimicrobial therapies and wound care applications.

P10 Exploitation of winemaking by-products from wineries of Lemnos island, Greece

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Grape cultivation is one of the most important agro-economic activities worldwide [1]. The global grape trade is valued at 9.4 billion euros, while the global wine trade is worth 37.8 billion euros [2]. During the winemaking process, Europe alone generates 14.5 million tons of by-products, including grape stems, pomace, and lees. These by-products have been studied across various countries and have been shown to be rich in bioactive compounds with antioxidant and other beneficial biological properties [1]. The valorization of these agro-industrial by-products aligns with the principles of the “3Rs” “Reduce, Reuse, and Recycle” core to the concept of circular economy [3].

The present study aims at exploiting the potential of wine-making waste from wineries in Lemnos island, in terms of chemical constituents and bioactivity. Several samples of grape stems and pomaces from four grape varieties, cultivated under conventional and organic methods, were studied. The extraction techniques employed included Ultrasound-Assisted and Accelerated Solvent Extraction, with the goal of optimizing the extraction parameters to maximize the yield of bioactive compounds. The optimized extracts were analyzed by HPLC-DAD, resulting in the quantification of 15 phenolic compounds, using analytical standards. Furthermore, their GC-MS analysis identified 27 volatile compounds. In addition, the extracts were assessed for their total phenolic content using the Folin-Ciocalteu reagent and for antioxidant activity using the DPPH assay, both demonstrating notable results.

The results highlight the considerable potential of winemaking by-products for use in functional food, nutraceutical and cosmetic applications, offering a valuable source of bioactive compounds with health-promoting properties.

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P11 Subcritical water extraction and fractionation: A green approach to recovering antioxidants and caffeine from black tea

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Tea (*Camellia sinensis*) is the second most consumed beverage after water, valued for its health benefits (antioxidants) and stimulating properties (caffeine) [1]. Black tea, a fermented variant, is widely available, including in dietary supplements and other products, but its manufacturing often relies on inefficient and environmentally harmful extraction methods [2, 3]. Subcritical water extraction (SWE) is a promising green alternative, enhancing yields and efficiency [4]. However, globally inconsistent methodologies lead to varying scientific assessments. This study optimized SWE to maximize biologically active compounds, using fractionated extraction (5 x 2 min, 2 x 5 min, 10 min). Furthermore, SWE extracts were compared with traditional tea preparation, water infusion (WI). Results showed that antioxidant activity and caffeine content increased with longer extraction times, particularly in the second 5 min or at 10 min fractions. The strongest antioxidant potential (DPPH IC₅₀ 16.23 µg/mL; ORAC IC₅₀ 6.43 µg/mL; TPC 650.55 mg GAE/g) and highest caffeine content (14.834 µg/mL) were found in the second 5 min fraction. Individual phenolic compounds were determined by LC-MS/MS. Our results also revealed that certain phenolics, such as chlorogenic acid, epicatechin, hyperoside, isoquercitrin, and rutin, require precise extraction timing to optimize yield, as the highest concentration occurred either within the first 5 min or in some of 2 min fractions. However, other compounds, such as catechin or gallic acid, showed highest concentrations in WI. These findings demonstrate that extraction time significantly affects final extract composition. Furthermore, as different compounds respond to different SWE conditions, further optimization of temperature and pressure is required to improve extraction efficiency.

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P12 Unveiling the Natural Bioactivity and Molecular Messaging of Plant Extracellular Vesicles in Plant-Fungi Interactions

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Plant extracellular vesicles (EVs) are natural nanocarriers enriched in bioactive molecules, playing key roles in intercellular communication during both defense responses and symbiotic interactions, [1, 2]. However, their molecular composition and functional roles in plant-fungi interactions remain poorly investigated. This study aimed to characterize the „SecrEVome“, the suite of bioactive molecules transported by plant EVs in interactions with beneficial (*Trichoderma* spp.) and pathogenic (*Botrytis cinerea*) fungi using tomato (*Solanum lycopersicum*) as a model.

EVs were isolated by differential ultracentrifugation (dUC) from both root exudates and apoplastic fluids. Specifically, for the tomato-*Trichoderma* interaction model, a split-root hydroponic system was employed to avoid fungal EV contamination. In the pathogenic model, EVs were collected from root exudates and leaves distal to the *B. cinerea* infection site to assess systemic responses. Additionally, tomato hairy root (hr) cultures were used to produce EVs (hrEVs). Vesicle size and concentration were determined by nanoparticle tracking analysis (NTA), and morphology was confirmed by transmission electron microscopy

All EVs exhibited similar size profiles, with hrEVs providing the highest yields. Metabolomic profiling identified tomatine and tomatidineol as prominent components, suggesting an inherent antimicrobial activity. Proteomic analyses revealed enrichment of defense-related proteins in EVs upon *B. cinerea* infection. Ongoing miRNA profiling aims to uncover additional regulatory functions. This multi-omics study will provide a detailed molecular portrait of tomato EVs and unveil their role in modulating plant-microbe interactions. The findings also support the potential of EVs as bioactive carriers in sustainable crop protection strategies aligned with circular bioeconomy principles, promoting the valorization of plant biomass and by-products while offering prospects for human health applications.

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P13 Innovative microextraction of bioactive metabolites from *Cannabis sativa* L. aerial parts using eutectic solvents

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Cannabis sativa L. is a plant that has been used since ancient times for medicinal, textile and food purposes. From a chemical point of view, it is a very complex matrix consisting of several classes of specialized metabolites, including cannabinoids, terpenoids and flavonoids [1]. The metabolomic profile of the plant varies considerably depending on which part of the plant is considered. Research has traditionally focused mainly on cannabinoids, but there is growing interest in the potential synergistic bioactivity of the entire metabolome, emphasizing the importance of comprehensive profiling. A key challenge in this context is the extraction process, which has a decisive influence on the detection and quantification of bioactive compounds. Conventional extraction techniques often rely on large amounts of toxic and environmentally harmful organic solvents. However, recent advances in microextraction and in the development of greener solvents offer promising alternatives in line with regulatory requirements and public attention to environmental sustainability [2].

This study is focused on the characterization of hemp aerial parts (leaves and stems), rich in non-psychotomimetic cannabinoids and flavonoids [3]. Initially, ultrasound-assisted methanol extraction was used to isolate these compounds. To improve sustainability, the study explores eutectic solvents consisting of natural terpenoids, phenolic compounds, and choline derivatives in combination with ultrasound-assisted dispersive solid-liquid microextraction [4, 5]. This approach successfully replicates the chemical profile of conventional methods while improving analyte enrichment and reducing environmental impact. The results emphasize the potential of DES-based microextraction as a viable, environmentally friendly alternative for comprehensive metabolomic studies in *C. sativa*.

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P14 Unlocking the hidden value of *Camellia oleifera*: A cosmetic perspective on oil by-products

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Camellia oleifera seed oil has been extensively used in China for centuries, both in culinary traditions and cosmetic applications. The oil is extracted from the seeds of the *Camellia oleifera* tree, and its production generates several by-products. Among these, the *Camellia oleifera* fruit shells (COFS) and *Camellia oleifera* seed shells (COSS) are agricultural residues, while the *Camellia oleifera* seed cake (COSC) is a process-derived by-product obtained after oil extraction. One of the most effective strategies to significantly reduce the environmental footprint of camellia oil production is the valorization of these by-products.

The aim of this study is to explore and identify high value-added cosmetic applications for these by-products. The COFS were micronized to produce an ultra-fine powder with mattifying properties, suitable for use in cosmetic make-up formulations. Cellulose extracted from the COFS was used to create preliminary fibers, offering potential for textile applications. The COSS were dried, sterilized, and ground to be used both as a natural exfoliating agent in skincare products and as a biobased alternative to plastic in cosmetic packaging. Finally, proteins extracted from COSC were hydrolyzed to generate peptides with demonstrated anti-aging biological activity on the skin. Additionally, saponins isolated from COSC exhibited stabilizing and emulsifying properties, making them valuable for incorporation into skincare formulations. Traditionally, *Camellia oleifera* is cultivated solely for its oil used in cosmetics. However, by adopting a more sustainable and resource-efficient model, the same crop can provide not only this primary ingredient but also six additional cosmetic ingredients from its by-products.

P15 Biotechnological value of *Moringa oleifera* Lam. (*Moringaceae*) seedcake: A glucomoringin-rich byproduct for the development of a sustainable biopesticide

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Moringa oleifera Lam. (*Moringaceae*) is a multifunctional tree cultivated in all tropical countries for its nutritional properties and a variety of end uses. Noteworthy, *M. oleifera* seed is largely exploited for its edible oil, producing a seedcake biomass as a byproduct of oil extraction [1, 2]. The seedcake is rich in 4-(α -L-rhamnosyloxy)benzyl glucosinolate (glucomoringin), the chemical tag of the plant and the precursor of 4-(α -L-rhamnosyloxy)benzyl isothiocyanate (moringin) [3]. The objectives of this work were the extraction and isolation of pure glucomoringin on a multigram scale, and the evaluation of its antifungal potential for the control of infections by *Fusarium oxysporum* f. sp. *lycopersici*, the causal agent of wilting on tomato [4]. The commercial seedcake PKM-2 (100 g) showed a remarkable glucomoringin content (13.9%), determined according to the ISO 9167:2019 method [5]. After extraction with boiling water, pure glucomoringin (4.2 g) was obtained by a two-step chromatographic process. Glucomoringin and moringin solution freshly prepared by myrosinase-catalyzed hydrolysis of the precursor were evaluated for antifungal action *in vitro*, as well as *in planta*. Spore germination and mycelial development of *F. oxysporum* f. sp. *lycopersici* were significantly inhibited *in vitro*, following exposure to moringin (range 1–0.001 mM), compared to treatments with glucomoringin, which didn't differ significantly. Similarly, the coating of tomato seeds (var. crovarese) with moringin (0.1 mM) was effective in reducing significantly (~70 % ca) the severity of the disease in post-emergence. This study highlighted the potential biotechnological value of *M. oleifera* seedcake for the development of a sustainable biopesticide.

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P16 Biodiversity and sustainability in Cilento: The link between resilient agriculture and the local economy

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The Cilento Biodistrict combines agricultural sustainability and biodiversity conservation. Despite its reputation for organic agriculture, climate change necessitates an in-depth analysis of local dynamics, with a focus on the socio-economic implications for the community. This analysis integrates various studies on the role of biodistricts in addressing environmental challenges and their impact on the well-being of local populations. The study examines biodistricts as models of sustainable development, exploring the relationship between climate change, biodiversity, and socio-economic well-being. It focuses on how organic agriculture practices can mitigate the effects of climate change, protect natural resources, and generate economic benefits by strengthening the socio-economic resilience of vulnerable areas. The findings show that biodistricts, such as Cilento, mitigate the effects of climate change through sustainable agricultural practices. The reduction of pesticides and chemical fertilizers, along with the enhancement of local resources, has improved soil quality and preserved biodiversity (► Fig. 1). However, climate change has also had significant socio-economic impacts, affecting agricultural production and increasing the vulnerability of rural populations.



► Fig. 1 Biodistricts in Italy by 2023

The results indicate that biodistricts can play a crucial role in adapting to climate change by enhancing both ecological and economic resilience in these areas. It is vital to adopt supportive policies that facilitate the transition to sustainable agricultural practices and provide socio-economic support to local communities. An integrated approach that links agriculture, biodiversity, and adaptation policies is essential for the long-term sustainability of vulnerable territories.

P17 Sustainable valorisation of *Vitis vinifera* cv. Lambrusco pomace: Enhancing bioactive compounds and antioxidant activity

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Italy, one of the world's top wine producers, generates significant quantities of grape pomace—still rich in bioactive compounds, mainly polyphenols [1, 2]. *Vitis vinifera* cultivar Lambrusco pomace, a residue from vinification processes, represents a key subject of study related to a broader project focused on specific contexts of the 2030 Agenda, in particular on the sustainable valorisation of agro-industrial by-products for health and agronomic applications (PERCIVAL, Licofarma 2018).

Fresh pomace was immediately extracted using 60 % EtOH with sonication, yielding 6.34 %. Additional extractions were performed after 24 and 48 hs on pomace stored at room temperature and at 4 °C. While refrigerated samples maintained yields above 6 %, room temperature storage resulted in a progressive reduction to 4.59 % after 48 hs. Extracts analysis revealed a significant decrease in sugar content, from 26.69 % in fresh pomace to 5.61 % after 48 hs at room temperature. However, polyphenol content, assessed via Folin-Ciocalteu, showed no statistically significant difference ($p > 0.05$) in the time range considered, in parallel with a strong antioxidant activity (all extracts gave an IC₅₀ between 10 and 20 µg/mL against DPPH radical). These results are in line with previous studies on the same matrix [3].

These findings highlight the potential of *Vitis vinifera* cv. Lambrusco vinification byproducts for health, cosmetic, and nutraceutical applications [4], as well as demonstrating that proper storage can affect pomace composition, reducing sugars, while maintaining or enhancing bioactive components.

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P18 How could climate change impact the phytochemical quality of medicinal and aromatic plants? A case study of *Lavandula* species products

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Several *Lavandula* species are medicinal and aromatic plants with economic value for phytopharmaceuticals, food, cosmetics, and aromatherapy. The genus *Lavandula* has 39 species, and fine lavender oil, spike lavender oil and lavandin oil are the main ones cultivated for essential oils [1]. Fine lavender oil (i.e., *Lavandula angustifolia* Mill.) is the primary species due to its pharmacological properties and clinical evidence [2]. It is considered safe (GRAS) to be consumed in oral dosage or topical forms without serious adverse effects [1]. Sillexan® is a fine lavender oil used to treat mild anxiety [3, 4]. It can also be found as a natural health product or food supplement. The phytochemical analysis showed that 63 % of products contain less or none of the active metabolites/marker compounds (e.g., 1,8-cineole, linalool, and linalyl acetate) [1]. Considerable variability was also observed between products at the times of 20.7, 46.5, and 46.8 minutes, corresponding to 1,8-cineole, linalool, and linalyl acetate, respectively [1]. Our findings show chemical composition of finished products can vary, impacting pharmacological and clinical activities [1]. However, there

are core challenges and consequences associated with growing and sustaining the primary materials of *Lavandula* species linked to climate change, such as altered flowering patterns, increased pest pressure and diseases, water stress and drought and social and economic consequences, particularly with the potential shifts in the cultivation regions [5] (► Fig. 1). Chemical variability is linked to various factors, so understanding the potential impact of climate change on lavender product quality is crucial, both for this important medicinal crop and its products, and for the wider industry.



► Fig. 1 Core challenges and consequences associated with growing and sustaining the primary materials of *Lavandula* species linked to climate change

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P19 Arbuscular mycorrhizal fungi as regulators of the impact of climate change on the cultivation and metabolome of the medicinal and aromatic plant *Coleus amboinicus* Lour

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Climate change will threaten the ecosystems on which people depend for their health. This phenomenon will be particularly pronounced for populations in developing countries who rely on medicinal plants (MAPs) as their first-line medicines [1]. Environmental factors have been shown to influence both the growth and the molecular composition of MAPs. Increases in atmospheric CO₂ and temperature or drought are major climatic perturbations that have variable consequences [2]. Arbuscular mycorrhizal fungi (AMF) can induce changes in specific metabolites of MAPs by activating metabolic pathways. AMF are obligate symbionts with great potential to improve plant growth through a variety of mechanisms, including increased mineral nutrition, water uptake, drought and disease resistance and plant productivity [3]. *Coleus amboinicus* Lour. is a semi-succulent plant of the Lamiaceae family with a pungent oregano-like odour that is widely cultivated in the tropics and whose leaves have many uses in traditional medicine [4]. To study the impact of climate change on the growth and metabolome of *C. amboinicus*, we performed a culture experiment in a controlled environment (CO₂ rate, temperature, differentiated irrigation) by inoculating or not the cultures with AMF. Untargeted metabolomic approaches based on mass spectrometry were used to identify new patterns in biotic and abiotic interactions. Our results show that water conditions have a significant impact on growth, extraction yields and molecular composition. Volatile molecules are over-expressed under lower water conditions and in the presence of AMF, while the composition of non-volatile compounds changes differently depending on the metabolite family considered in response to the same conditions.

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P20 Understanding of nitrogen cycling in lichens of the genus *Cladonia* by NMR metabolomics

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Understanding nitrogen dynamics in lichens, while providing insights into lichen physiology and symbiosis, can also be important in assessing the impact of human footprint on ecosystems. In this work, we studied four species of *Cladonia* Hill. (*C. foliacea*, *C. polycarpoides*, *C. rangiformis*, *C. rei*), a genus of fruticose lichens with cosmopolitan distribution which provide relevant biomass and ecosystem services in rare and endangered habitats [1, 2]. Using NMR metabolomics, we found out metabolic markers of seasonal variation common to the four species, providing a key to understanding how nitrogen and carbon are mobilized across seasons. The developed OPLS model, with y = temperature

gradient over the year (dT), showed that as dT decreases, glutamine and the sugar alcohol arabinitol increase, whereas as dT increases, there is an increase in threonine, glucose, sucrose, and trehalose, together with another sugar alcohol found for the first time in lichens. This key and rare metabolite was identified (by 2D NMR and ToF-MS) as β glucopyranose(1 $\rightarrow$ 2)mannitol. It is reasonable to hypothesize that nitrogen is assimilated into glutamine during winter and released in spring-summer, favoring lichen catabolism and reproductive activity. The observed decrease in glutamine, coupled with threonine accumulation, suggests a selective channeling of nitrogen resources toward threonine biosynthesis, possibly due to regulatory shifts. The observed seasonal cycle – from glutamine and arabinitol accumulation to peaks in threonine and β -glucopyranose(1 $\rightarrow$ 2)mannitol, followed by a winter reset – suggests a highly integrated nitrogen-carbon regulatory mechanism that is likely tuned to environmental changes, with β -glucopyranose(1 $\rightarrow$ 2)mannitol as the most important carbon vehicle.

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P21 Synthesis and application of hydrochar from artichoke as an innovative product to increase soil fertility and act as plant biostimulant

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Soil degradation, which is mostly due to human over-exploitation and climate change, severely impacts on global food production and terrestrial biodiversity. The hydrochar (HC) is an innovative and still unexplored product, resulting from the hydrothermal carbonization of organic biomass, and potentially acting as improver of soil fertility. Therefore, artichoke biomass was processed with subcritical water to produce several HC lines which were investigated as both ameliorative of soil quality and plant biostimulant. HCs were developed adopting two temperature and pressure conditions (180 °C and 10 Bar; 215 °C and 20 Bar) and evaluating different types of artichoke wastes (stems and bracts, either alone or combined in a ratio 30:70 w/w and *viceversa*). Solid hydrochars (HC_sol) showed good levels of nitrogen (5–14 g/kg) and ash (1.3–6%), along with strong waterholding capacity. HC_sol also exhibited the ability to adsorb heavy metals (cadmium and lead) thus paving the way to environmental applications. The liquid phases (HC_liq) showed an acidic pH (3.5–4) and high levels of phosphorous and phenols serving as plant biostimulants. When applied at the proper concentrations, several lines of HC_liq exerted an efficient biostimulant action on cress and radish seeds, up to elicit a response 55 % higher than the control. For cress, the best HC-liq was that obtained by 100 % stems at 215 °C, while, for radish, the more efficient HC_liqs were those achieved by 70 % bracts at 215 °C and 100 % stems at 180 °C. Our outcomes proved that HC is a virtuous, green and promising technique to recycle artichoke wastes.

P22 Maximizing the potential of artichoke by-products: developing functional bakery products with nutritional and bioactive extracts

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In the pursuit of sustainable innovation in the food industry, this study explores the valorization of artichoke (*Cynara scolymus*) by-products through their incorporation into functional bakery products. In collaboration with Dolciaria Monardo, we aim to develop a new line of sweet baked goods enhanced exclusively with extracts derived from underutilized parts of the artichoke plant, such as bracts and stems.

Three distinct extracts were obtained and characterized for their nutritional and biofunctional properties:

- Inulin-rich extract, offering prebiotic and dietary fiber enhancement;
- Chlorogenic acid-rich extract, known for its strong antioxidant activity;
- Cynarine-rich extract, associated with hepatoprotective effects.

These extracts are being integrated into bakery formulations to create products that meet sensory and technological standards while also providing added health benefits. The goal is to combine nutritional fortification with appealing flavor and texture profiles, while contributing to the circular economy through the upcycling of agricultural by-products. This approach demonstrates how the targeted use of bioactive plant fractions can lead to innovative, health-oriented bakery items with clear consumer and environmental value. The semi-industrial extraction process is carried out by Vitrosele, a leading grower and producer of artichoke-based food products, specializing in the sustainable valorization of artichoke by-products.

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P23 Exploring the therapeutic properties of Centauri® honey: Antimicrobial and anti-skin-related enzymes study

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Centaury® Cave Nymph Honey is a distinctive natural product collected from Turkey's alpine regions, made by bees from endemic medicinal plants such as *Centaurea solstitialis*. Harvested from an untouched environment, minimizes contaminants, preserving its bioactive compounds.

Previous studies have reported its physicochemical characteristics and promising anti-inflammatory activity [1]. Building upon these findings, the present study aimed to further explore the bioactive potential of one Centaury® honey sample, collected in Turkey (August 2022) (A) and two additional supplemented samples with propolis (AP) and gold (AG) evaluating its antimicrobial activity and its inhibitory effects on skin-related enzymes

Antimicrobial activity of these three samples was evaluated through minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) assays. The results indicated that different all Centaury® honey samples (A, AP, and AG) exhibit promising antimicrobial effects against all tested strains. Samples A demonstrated effectiveness particularly against *E. coli*, showing inhibition at concentrations as low as 0.05 % (v/v). The enriched variants, AP and AG, exhibited amplified antimicrobial effects, especially against Gram-positive bacteria (*S. aureus* and *E. faecalis*) and fungal strains (*S. cerevisiae* and *C. albicans*), with minimum inhibitory concentrations (MICs) ranging from 0.05 % to 0.781 % (v/v). All Honey samples showed in vitro inhibitory effects on skin-related enzymes, where the honey supplemented with propolis AP exhibited the highest enzymatic inhibitory potential (44 % to 74 %). Centaury® honey, especially when enriched with propolis (AP) or gold (AG), demonstrates promising antimicrobial and anti-enzymatic activity, highlighting its potential for cosmetic applications through effective inhibition of skin-related enzymes.

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P24 Open platform for ethnopharmacology InovafitoBrasil: A new collaborative approach for drug discovery from medicinal plants in developing countries

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InovafitoBrasil is a non-profit, open-access platform designed to catalyze innovation in life sciences through the sustainable use of biodiversity and traditional knowledge in megadiverse developing countries. Despite hosting the majority of the planet's botanical diversity, these nations remain underrepresented in the global herbal medicinal products (HMPs) market. The platform addresses this disparity by offering a structured innovation framework based on NASA's Technology Readiness Levels (TRLs), supporting the development of high-quality HMPs in accordance with international regulatory standards: ANVISA, EMA, FDA, WHO, ICH. [1]. It also provides comprehensive resources

on access and benefit-sharing (ABS) policies, including interpretative guidance on the Nagoya Protocol and Brazilian regulations governing genetic resources. Developed through a public-private collaboration involving leading Brazilian pharmaceutical companies, the national health authority ANVISA, and the Oswaldo Cruz Foundation (Fiocruz), InovafitoBrasil currently supports over 80 projects and more than 600 registered stakeholders. The platform is publicly available on: <https://inovafitobrasil.com.br/en/index.html>. By bridging scientific, regulatory, and commercial barriers, InovafitoBrasil promotes equitable bioeconomic development and serves as a replicable model for integrating biodiversity into national innovation strategies. Its potential formal adoption into Brazil's drug development policies signals a paradigm shift toward more inclusive, sustainable, and biodiversity-centered approaches to global health innovation.

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P25 Enhancing green extraction techniques for the valorization of artichoke by-products: A starting point for industrial scalability

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The artichoke (*Cynara cardunculus* var. *scolymus* L.), a plant in the Asteraceae family, is traditionally cultivated for its large, immature inflorescences, known as capitula. This plant is a valuable source of bioactive compounds, including flavonoids, caffeoyl derivatives and inulin, a fructan polymer recognized for its prebiotic effects [1]. The remaining parts of the plant (approximately 80-85 % of the total biomass) [2] are discarded during cultivation and industrial processing, resulting in significant amounts of waste. This research focused on the efficient recovery of valuable metabolites from artichoke by-products, including stems and leaves, by exploring innovative and eco-friendly extraction methods, specifically pressurized hot water extraction (PHWE) and ultrasound probe extraction (USE probe) [3].

Optimal extraction conditions were established, based on Design of Experiments (DoE), to minimize the number of trials, thereby increasing efficiency and reducing resource consumption. On this basis, this work represents a starting point for a scalable industrial extraction process. The inulin content, along with the concentration of specialized metabolites, was evaluated using an enzymatic/spectrophotometric method and by UHPLC-Q-Orbitrap-HRMS analysis, respectively. The results revealed that artichoke leaves are particularly rich in secondary metabolites, such as caffeoylquinic acids, flavonoids, and sesquiterpene lactones. Additionally, the stem by-product may represent a valuable source of inulin fiber. Hence, artichoke by-products can be considered a source of health-promoting ingredients for food supplements and functional food.

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P26 Evaluation of subcritical water extraction for recovery of bioactive compounds from silver fir (*Abies alba*)

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The chemical richness of silver fir (*Abies alba*), particularly its bark and branches, makes it a valuable source of polyphenols, lignans, and terpenes with antioxidant and therapeutic potential [1, 2].

This study aimed to systematically evaluate various extraction techniques, including subcritical water extraction (SWE), supercritical CO₂ extraction, high-pressure ethanol extraction, and conventional water extraction, with a focus on optimizing yield and bioactivity. SWE was performed at constant pressure (100 bar) and varying temperatures (70–200 °C) to examine temperature-dependent extraction efficiency. The SWE extract from bark at 100 °C (SWE-BA-100) exhibited the highest total polyphenol content (73.8 mg GAE/g), lignan concentration (273.2 µg/mL of secoisolariciresinol), and antioxidant activity (DPPH: 24.2 mg GAE/g; ABTS: 32.0 mg GAE/g). GC-MS analyses confirmed a complex phytochemical profile including monoterpenes and sesquiterpenes. *In vitro* cytotoxicity assays on HaCaT and Caco-2 cells showed no toxic effects, supporting the extracts' safety. However, SWE-BA-100 significantly inhibited cell migration, likely due to its high lignan content, indicating that while the extract is safe, it may modulate wound healing processes.

The study demonstrates that SWE, particularly at moderate temperatures, is an effective, green technology for recovering bioactive compounds from silver fir. These findings support its application in developing functional ingredients for dermatological and nutraceutical products and highlight the need for compositional fine-tuning to balance extraction efficacy and biological response.

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P27 Optimization of green extraction techniques of *Ceratonia siliqua* leaves compounds

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The growing demand for sustainable bioactive compounds, sourced from renewable plant materials, is pushing the development of eco-friendly extraction methods. These compounds, derived from plant sources and agrifood by-products, offer health benefits and promising applications in the food, pharmaceutical, and cosmetic industries, providing a safer alternative to synthetic chemicals with lower environmental impact. Therefore, developing efficient extraction methods for these metabolites is crucial for enhancing product quality and supporting sustainable practices, contributing to European sustainability initiatives and the United Nations' Sustainable Development Goals [1].

Green extraction technologies, such as Pressurized Hot Water Extraction (PHWE) and Ultrasound Probe Extraction (USEprobe) have emerged as highly effective alternatives to conventional methods [1]. These techniques align with the six principles of eco-extraction, which focus on minimizing solvent use, energy consumption and process time, while maximizing the recovery of bioactive compounds. Moreover, these methods offer scalability, making them

ideal candidates for industrial applications where efficiency and sustainability are paramount [2].

In this context, this research focused on the development and optimization of green extraction processes for bioactive recovery from *Ceratonia siliqua* leaves (CSL), untapped biomass of a typical Italian crop mainly cultivated for its fruits, that have shown promising health-promoting potential.

Response surface methodology (RSM) was utilized to optimize extraction conditions by simultaneously analyzing the effects and interactions of multiple parameters. This approach, based on Design of Experiments (DoE), allowed the determination of optimal conditions with a minimal number of experiments, enhancing efficiency and reducing resource usage.

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P28 Is there any influence of microplastics in the soil on the qualitative characteristics of basil (*Ocimum basilicum*)?

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Basil (*Ocimum basilicum*, *Lamiaceae*) is a medicinal herb rich in polyphenols and bioactive compounds, valued for its wide range of uses in both humans and animals. The trial was conducted using *Ocimum basilicum* var. *Violetto*. Four treatments, each with four replications, were applied: a control (C) and three experimental groups (E1–E3), with the addition of 30 mg/kg of PVC microplastics (particle size 63–125 µm). In the E2 group, the fungus *Trichoderma atroviride* (spore concentration of 10⁸ per 10 kg of substrate) was added to the substrate. In the E3 group, a mycelium of *Sclerotinia sclerotiorum* was added.

The pots were kept in a growth chamber at 18–25 °C (night-day regime) and 60 % humidity, with a 10–14 h photoperiod, for four weeks. Basil leaves were collected to examine total antioxidant activity using the ferric reducing antioxidant power (FRAP) assay [1]. Vitamin C was quantified via its reaction with 2,4-dinitrophenylhydrazine (DNPH) after oxidation with dehydroascorbic acid, forming a colored complex measured at 520 nm.

Nematodes were isolated from the substrate using the Baermann funnel method, counted under a stereozoom magnifier, and then mass-fixed on microscope slides. Regarding antioxidant activity, plants from the C and E2 groups showed significantly higher ($P < 0.05$) FRAP values (32.87 and 33.33 mmol FeSO₄ per 1 mg of fresh leaves, respectively) compared to the E1 and E3 groups (23.89 and 23.75 mmol FeSO₄, respectively).

In terms of vitamin C content, the C group (167.74 µg/g) had a significantly higher concentration ($P < 0.05$) than the E1 (131.49 µg/g) and E3 (110.81 µg/g) groups. Nematode abundance per 50 g of substrate was as follows: C – 3197, E1 – 1264, E2 – 1008, and E3 – 828, with a 99 % prevalence of the genus *Eucephalobus* in all groups. The initial results indicate that the presence of microplastics significantly reduces basil quality, while *Trichoderma atroviride* enhances the plant's resistance [2].

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P29 Correlation analysis between growth characteristics of wild-simulated ginseng and soil bacterial communities living in rhizosphere soils

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Bacterial communities can influence the growth and active compounds of plants. In previous studies, the influential endophytes were obtained from wild-simulated ginseng (WSG, *Panax ginseng* C.A. Meyer) and their biochemical and physiological activities were measured. Some bacterial strains enhanced the growth of WSG. The aim of this study was to identify the bacterial strains and to investigate the relationship between the growth of WSG and bacterial communities. The most effective bacterial strain (75) was identified as *Pseudomonas frederiksbergensis*. When the bacterial strains were treated in the WSG experimental soil, the relative abundance of Pseudomonadales order was significantly increased by the treatment of strains 75 and 77. However, the relative abundances of Burkholderiales and Bacillales orders did not show significant differences with treatment of bacterial strains. In the analysis of relationships between WSG growth and relative abundances of bacterial communities, Pseudomonadales and Burkholderiales showed positive correlations with root length and dry weight, respectively. In the analysis of α -diversity, diversity decreased significantly in bacterial communities (77, 79, 81, 89), but increased in fungal communities (75, 210). In the analysis of β -diversity, bacterial and fungal communities did not show any significant differences. These results of this study showed that the soil bacterial community may influence the growth of WSG and its cultivation environments [1, 2].

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P30 Phytochemical insights and cytotoxicity on colon cancer cells of unisexual flower extracts from *Ceratonia siliqua* L. (Fabaceae)

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Ceratonia siliqua L. (carob) is an evergreen tree from the Fabaceae family, native to the Mediterranean and cultivated for its economic value [1]. The species is dioecious, with male and female flowers on separate plants, though hermaphroditic individuals exist. While the fruits are known for their polyphenol, flavonoid, carbohydrate, mineral, and protein content, the chemical composition of the flowers remains less explored [2].

This study assessed the phytochemical profile of two alcoholic extracts from female (FF) and male (MF) flowers of *C. siliqua* using UPLC/ESI/TQ-MS, evaluating their total polyphenol and flavonoid content, antioxidant properties, and cytotoxicity on healthy and cancerous cells. The analysis revealed a higher polyphenol than flavonoid content, with qualitative and quantitative differences

between extracts. MF exhibited significantly higher levels of polyphenols, gallotannins, and flavonoids, identifying 13 phenolic compounds compared to 7 in FF, including 2-digalloyl hexose, gallic acid, and quercetin.

Antioxidant activity was confirmed through DPPH, SOD-like, and Catalase-like assays, with FF showing higher Catalase-like and MF higher SOD-like activity. MTT tests demonstrated the extracts' safety on normal cells (HFF-1) and a dose-dependent cytotoxicity against cancer cells (CaCo-2). LDH release tests excluded necrosis as the primary cause of cell death. ROS and RSH level analysis indicated a 30-35% reduction in RSH content, suggesting glutathione depletion and cell cycle alteration.

For the first time, this study demonstrates that carob flowers are a source of phenolic compounds with antioxidant and cytotoxic activities, potentially mediated by GSH depletion. These findings support further research into the therapeutic potential of *C. siliqua* flower polyphenols.

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P31 Evaluation of the antiproliferative effect of enantiomers of carvone in association with silver complexes in skin cancer cell lines

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Skin cancer is a major global concern, with squamous cell carcinoma (SCC) and melanoma (CM) as prevalent subtypes [1]. Conventional drugs like cisplatin and 5-fluorouracil (5-FU) face efficacy and penetration challenges, highlighting the need for new therapies [2]. Monoterpenes such as carvone may improve transdermal drug delivery [3]. The combination of silver complexes with bioactive ligands derived from sulfonamides (Ag-1) and nucleobases (Ag-2) with carvone offers a promising strategy, uniting the anticancer effects of metallo-drugs with improved skin permeability for topical treatment [3]. Our study evaluated the *in vitro* antiproliferative effects of silver complexes [4, 5] and the enantiomers of carvone, isolated and in association. Results were expressed as concentration required to elicit 50% of cell growth inhibition (GI50) and as combination index reduction (CRI). After 48h-exposure, the Ag-1 and Ag-2 complexes exhibited antiproliferative activity, with GI50 < 4 μ M in FaDu, SCC-9 and SK-MEL-28 cells, while R-(-) and S-(+)-carvone were inactive (GI50 > 400 μ M). Synergistic effects (CRI < 1) were observed for the combination R-(-)-carvone (4 μ M)/Ag-1 in SCC-9 cells (GI50 < 1 μ M, CRI = 0.4) and R-(-)-carvone (4 μ M)/Ag-2 in FaDu (GI50 < 1 μ M, CRI = 0.7), SCC-9 and SK-MEL-28 cells (GI50 < 1 μ M, CRI = 0.5). The combination with S-(+)-carvone did not show any interaction (CRI > 1), evidencing the influence of stereoisomerism on the biological effect. These results suggest that R-(-)-carvone may enhance the antiproliferative effect of silver complexes in melanoma and squamous cell carcinoma, justifying further studies of this combination.

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P32 Evaluation of acetylcholinesterase and monoamine oxidase inhibitory activities of the aqueous extract and flavonoids of *Capparis cartilaginea* Decne

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Capparis cartilaginea Decne. (Capparaceae) is a medicinal plant with a long history of use in traditional medicine. Despite its ethnopharmacological relevance, its neuropharmacological properties remain underexplored. In our previous work, seven glycosylated flavonoids were isolated and structurally elucidated from the aqueous leaf extract, including derivatives of kaempferol, quercetin, and isorhamnetin [1]. In the present study, the neuropharmacological potential of the aqueous extract and selected flavonoids was evaluated through the inhibition of acetylcholinesterase (AChE), monoamine oxidase A (MAO-A), and monoamine oxidase B (MAO-B), enzymes associated with neurodegenerative disorders such as Alzheimer's and Parkinson's disease. The aqueous extract exhibited no significant inhibitory activity against AChE at concentrations ranging from 0.005 to 0.5 mg/mL. In contrast, both the extract and isolated flavonoids showed concentration-dependent inhibition of MAO-A and MAO-B, with a markedly greater effect on MAO-B. The inhibition was observed in the range of 0.01 to 2 mg/mL. Based on their selective MAO-B inhibitory profiles, four flavonoids were selected for enzyme kinetics analysis: kaempferol-3-O-rutinoside (nicotiflorin), quercetin-3-O-rutinoside (rutin), quercetin 3-(2G-rhamnosylrutinoside) (manghaslin), and isorhamnetin-3-O-rutinoside (narcissin). These compounds demonstrated inhibition constants (K_i) in the nanomolar range, indicating potent and selective inhibition of MAO-B. These findings suggest potential pharmacological value and support further research into their application in neurological disorders.

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P33 *In silico* approach on mechanisms of action by funicone-like fungal metabolites in Canine Coronavirus infection

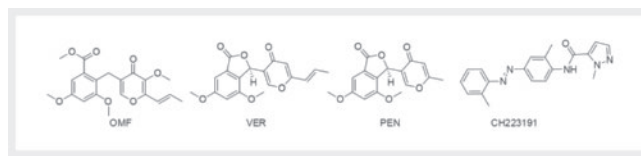
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The *alphacoronavirus* canine coronavirus type II (CCoV-II) usually causes moderate enteritis in dogs, but its recombinational ability has led to the emergence of variants that can provoke serious diseases in dogs and humans. Since 2005, pantropic strains of CCoV have emerged, causing lethal multi-systemic disease, especially in young puppies. Subsequently, harmful novel CCoV strains causing acute respiratory illness have been isolated from humans in several countries [1]. Polyketides, funicone-like compounds, are a homogeneous group of fungal secondary metabolites that are potential drug candidates due to their valuable properties, including antibiotic, antiviral, cytotoxic, and insecticidal bioactivities [2].

We demonstrated that 3-O-methylfunicone (OMF), vermistatin (VER), and penisimplicissin (PEN) (► Fig. 1), produced by the fungus *Talaromyces pinophilus*, have antiviral activity against CCoV in canine fibrosarcoma cells (A72) [3, 4]. Indeed, these compounds significantly increased cell viability and also caused a substantial decline in virus yield and in the levels of viral nucleocapsid protein. Interestingly, during treatment, a cellular defense mechanism was revealed in the downregulation of aryl hydrocarbon receptor (AhR) signaling, which is affected by CCoV infection [5].



► Fig. 1 Structure of 3-O-methylfunicone (OMF), vermistatin (VER), penisimplicissin (PEN), and CH223191

Here, we show a computational approach of Molecular Docking studies and structural modeling analysis. Using this approach, we investigated the molecular contacts involved in the stabilization of AhR-funicones complexes. The analyzed funicones interact with the same binding pocket recognized by the AhR inhibitor CH223191 (► Fig. 1), suggesting that they may be characterized by a similar recognition mechanism. Overall, these data suggest that funicones could represent potential innovative drugs, having AhR as a target for therapy against CoVs.

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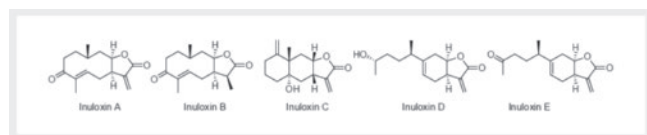
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P34 Antiproliferative activity of sesquiterpene lactones from *Dittrichia viscosa* (L.) Greuter: *In vitro* and *in silico* studies

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Plant-derived metabolites have provided promising leads in the search for safer anticancer chemotherapies. Among these, sesquiterpene lactones, predominantly present in the genus of the Asteraceae family, exhibit great structural diversity and a broad range of biological activities. *Dittrichia viscosa* (L.) Greuter, a plant species widespread in the Mediterranean region, represents a valuable source of natural bioactive compounds with potential applications in different fields [1]. In particular, five previously undescribed bi- and tricyclic sesquiterpene lactones, inuloxins A-E (► Fig. 1), and the known α -costic acid were isolated together from the aerial parts of *D. viscosa*2. The structures of these compounds were established by spectroscopic and chemical methods, while their absolute configuration was assigned by chiroptical and computational methods. Inuloxins A-E already showed interesting biological properties such as phytotoxic, acaricidal, and antileishmanial activities, suggesting their potential use in diverse applications [4]. This study aimed to evaluate their antiproliferative potential *in vitro* and identify their potential molecular targets *in silico* through network pharmacology and molecular dynamic simulation approaches, uncovering insights into their mechanisms of action and therapeutic potential. Furthermore, a new protocol for the efficient purification of bioactive compounds from *D. viscosa* has been introduced. Specifically, a solid-phase extraction (SPE) technique has been employed, reducing both the number of purification steps and the reliance on organic solvents.



► Fig. 1 Chemical structures of inuloxins A-E.

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P35 Antiviral potential and chemical characterization of secondary metabolites from *Strophanthus boivinii* active against dengue and Zika viruses

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Dengue virus (DENV) and Zika virus (ZIKV), two closely related orthoflaviviruses transmitted by Aedes mosquitoes, continue to pose major public health threats in tropical regions. [1] In Réunion Island, dengue epidemics have become recurrent, with 1,265 cases reported during the first half of 2024. Despite this burden, no effective antiviral treatment is currently available against ZIKV or DENV [2].

As part of a large-scale screening effort of 1,200 plant extracts from Malagasy endemic flora, we identified 10 extracts with significant antiviral activity. Among them, *Strophanthus boivinii* emerged as a promising candidate. We investigated its antiviral potential against ZIKV, using a molecular recombinant infectious clone expressing GFP (ZIKVGFP) [3], in human hepatoma (Huh7.5), alveolar epithelial (A549), and kidney epithelial (Vero E6) cell lines. The most active fraction (F19) showed IC₅₀ values of 0.01, 0.004, and 0.05 µg/mL, respectively, and CC₅₀ values > 2, 0.25, and > 100 µg/mL, corresponding to selectivity indices of > 200, 62.5, and > 2000, highlighting an excellent therapeutic window. Time-of-drug addition assays indicated that the active fraction acts during early stages of viral replication, likely interfering with viral translation or transcription processes. UPLC-HRMS² analysis coupled with molecular networking revealed the presence of cardenolide-type molecules in the active fraction. Ongoing work aims to isolate and identify the specific bioactive compounds responsible for the antiviral activity.

Our findings highlight the potential of *Strophanthus boivinii*, a Malagasy endemic plant, as a source of antiviral lead compounds and reinforce the value of biodiversity hotspots for natural antiviral drug discovery.

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P36 Protective effect of sinapaldehyde (SA) in alleviating neuroinflammation in lipopolysaccharide-induced BV-2 cells and in MPTP-stimulated in zebrafish

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Neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, and Huntington's disease are caused by the progressive degeneration and damage of nerve cells [1]. In our previous study, we confirmed the anti-inflamma-

tory activity of macrophages against SA [2], so in this study, we would like to investigate the neuroprotectiveness of SA using microglial cells and MPTP-induced zebrafish models. *In vitro*, BV2 cells were co-treated with SA and LPS to confirm inflammation-related protein and mRNA expression. SA showed activity inhibiting the production of NO and cytokines including TNF- α and IL-6 in LPS-stimulated BV2 cells. Protein expression levels of COX-2 and iNOS stimulated with LPS were also decreased by SA. On the other hand, the amount of COX-1 protein level remained unchanged. In addition, SA significantly down-regulated the mRNA expression of inflammatory genes, such as TNF- α and IL-6, against LPS stimulation. Furthermore, SA also suppressed the ERK and JNK phosphorylation, inhibited the activation of the NLRP3 inflammasome, and reduced the production of intracellular and mitochondrial ROS by LPS-induced BV2 cells. To evaluate the SA exercise effect, we used a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride (MPTP)-induced zebrafish model. MPTP-induced dysfunction of zebrafish was alleviated by SA. These results demonstrate that SA exhibits anti-inflammatory and antioxidant activity by inhibiting the MAPK signaling pathway, NLRP3 inflammasome activation, and ROS production. Moreover, SA alleviated abnormal response due to MPTP-induced neurotoxicity *in vivo*. This suggests that SA is a candidate that can inhibit various neurodegenerative disease progression.

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P37 Antioxidant and antiproliferative activity of two cultivars of *Coffea arabica* L. under different post-harvest conditions

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Coffee is the second most consumed beverage worldwide, with Brazil being the largest producer and exporter. Espírito Santo is the second-largest coffee-producing state, with a significant portion of its economy dedicated to this activity. *Coffea arabica* L. has the highest commercial value and is the most exported species. Beyond its economic importance, coffee is recognized for its pharmacological properties attributed to bioactive compounds with antioxidant and anticancer activities. This study evaluated the ethanolic extracts of two *C. arabica* cultivars, ‘Catuaí Vermelho IAC 99’ and ‘Catuaí Amarelo 2SL,’ following different post-harvest processing methods. Antioxidant activity was assessed using ABTS and DPPH assays, while antiproliferative activity was evaluated by MTT assay in human lymphocytes and tumor cells (Sarcoma-180). The results showed that extracts from dried and milled beans exhibited the highest antioxidant activity, suggesting that these methods are the most effective in preserving bioactive compounds. Conversely, roasting negatively impacts coffee’s medicinal potential by reducing bioactive compounds and antioxidant capacity [1, 2]. The MTT assay in human lymphocytes revealed a mitogenic effect, whereas all extracts induced cell death in tumor cells, with this effect being more pronounced post-roasting. These findings suggest that post-harvest processing, particularly roasting, alters the composition of bioactive com-

pounds, such as chlorogenic acid and trigonelline, as previously observed in other experiments by our research group, impacting the antioxidant and antiproliferative properties of *C. arabica*.

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P38 Triterpenes from Chios mastic gum as dual ROR γ and TGR5 modulators

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The retinoic acid receptor-related orphan receptor γ (ROR γ) and the G-protein-coupled bile acid receptor (TGR5) regulate Th17 cell differentiation and metabolic and immune functions, respectively [1, 2]. Their dual modulation may provide a novel therapeutic approach for inflammatory diseases [3]. Chios Mastic Gum (CMG), the exudate of *Pistacia lentiscus* var. *chia* (Anacardiaceae), is rich in triterpenes like masticiadienoic (MNA) and isomasticiadienoic (IMNA) acid [4, 5].

Given their structural similarity to known ROR γ and TGR5 ligands, we hypothesized that these compounds may act as modulators of these receptors.

An extract containing a mixture of MNA and IMNA, along with the purified compounds, were tested. TGR5 activity was assessed using a CRE-Luciferase reporter gene assay in TGR5 HEK EPAC cells, which express an EPAC (exchange protein directly activated by cAMP)-based FRET biosensor. ROR γ activity was evaluated with a ROR γ -Gal4 luciferase reporter assay in HEK293 cells. The extract and MNA were active on both receptors. Both 24E-IMNA and 24Z-IMNA activated TGR5, but only the Z configuration was active on ROR γ . Potencies, determined by nonlinear regression, ranged from nanomolar to low micromolar concentrations. Additionally, luciferase assays confirmed that these compounds showed no activity on other nuclear receptors, including LXR α , FXR, and VDR. Cytotoxicity was assessed via a resazurin conversion assay, indicating no toxicity in HEK293 cells at 1–10 μ M concentrations.

These preliminary results suggest that these compounds may modulate ROR γ and TGR5, warranting further investigation. Future studies will include NF- κ B assay and RT-qPCR in functional cell models to further explore their therapeutic potential.

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P39 Potential of *Salvia* extracts as natural insecticides against the tomato leaf miner, *Tuta absoluta*

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The tomato leaf miner, *Tuta absoluta*, is a major threat to solanaceous crops, particularly tomatoes, and can cause yield losses of up to 100 %. The rapid spread of the pest and increasing resistance to conventional insecticides highlight the need for alternative control methods [1, 2]. Considering these challenges, we evaluated the insecticidal and antifeedant activities of dichloromethane surface extracts from four *Salvia* species (*S. buchanani*, *S. corrugata*, *S. discolor*, and *S. namaensis*) against *T. absoluta* larvae using a leaf-dip bioassay protocol (IRAC susceptibility test method) with few modifications. LC-MS analysis revealed that the extracts were rich in abietane-type diterpenes and highly methoxylated flavonoids, which are known for their bioactive properties. Preliminary screening at a concentration of 2.5 mg/mL revealed *S. discolor* to be the most effective, demonstrating statistically higher larval mortality than the commercial insecticide Spinosad. Encouraged by these findings, we further evaluated the effects of the species at varying concentrations (0.16, 0.31, 0.62, 1.25, and 5.0 mg/mL). *S. discolor* demonstrated remarkable potency, with an LC50 of 0.013 mg/mL. Additionally *S. discolor* consistently induced strong feeding inhibition across all tested concentrations, suggesting its potential as a possible natural alternative to synthetic insecticides. Future studies will be carried out to investigate the compounds responsible for these effects.

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P40 Antibacterial activity of agrimony and oregano extracts: Isothermal microcalorimetry and LCMS analysis

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Agrimonia eupatoria (agrimony) and *Origanum vulgare* (oregano) are widely used traditional medicinal plants with notable antibacterial and wound-healing properties. This study aimed to evaluate the antibacterial activity of a 50 % methanolic extract of agrimony and an aqueous extract of oregano in various combinations (3:1, 1:1, 1:3). The antibacterial effects were assessed using a microdilution assay against the following bacterial strains: *Staphylococcus aureus* ATCC 29213 MSSA (MIC 2.5–5 mg.mL⁻¹), *S. aureus* ATCC 43300 MRSA (MIC 2.5–5 mg.mL⁻¹), *Proteus mirabilis* ATCC 29906 (MIC 2.5–5 mg.mL⁻¹), *Escherichia*

coli ATCC 25922 (MIC > 20 mg.mL⁻¹), *Enterococcus faecalis* ATCC 29212 (MIC 5–10 mg.mL⁻¹), and *Pseudomonas aeruginosa* ATCC 27853 (MIC 10–20 mg.mL⁻¹). The metabolic activity of MRSA and *P. aeruginosa* was further examined using isothermal microcalorimetry (IMC), which enables real-time monitoring of bacterial metabolism and a deeper understanding of antibacterial effects. Analysis of IMC thermograms and main parameters (heat flow, total heat, time to peak) revealed a synergistic effect: oregano effectively inhibited MRSA growth, while agrimony suppressed *P. aeruginosa*. LC-MS analysis identified major bioactive compounds in the extracts, including rosmarinic acid, oreganol A, and luteolin-7-diglucoside in oregano, and catechin, quercetin, apigenin, and luteolin glycosides in agrimony. These findings suggest that the combination of polyphenol-rich agrimony and oregano extracts offers a promising antibacterial strategy, particularly against MRSA and *P. aeruginosa*, common pathogens in chronic skin wounds.

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P41 Flavonoid-mediated inhibition of NLRP3 inflammasome activation: Anti-inflammatory insights from *Capparis cartilaginea* Decne

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Inflammation is a vital immune response, with the NLRP3 inflammasome playing a key role in mediating inflammatory activation. Dysregulation of NLRP3 is associated with diseases such as gout and type 2 diabetes, making it a promising therapeutic target. This study investigates the inhibitory potential of flavonoids isolated from the tea of *Capparis cartilaginea* Decne leaves, a plant widely consumed for its health benefits and cultivated in the Al-Taif region of Saudi Arabia. The identified compounds include quercetin 3-O-(2G-rhamnosylrutinoside) (Manghaslin), quercetin 3-O-neohesperidoside (Q3-Neo), quercetin 3-O-rutinoside (Rutin), kaempferol 3-O-(2G-rhamnosylrutinoside) (Clitorin), kaempferol 3-O-neohesperidoside (K3-Neo), kaempferol 3-O-rutinoside (Nicotiflorin), and isorhamnetin 3-O-rutinoside (Narcissin). *In silico* analysis, including molecular docking and molecular dynamics (MD) simulations, as well as *in vitro* assays such as ELISA and Western blotting, were conducted to explore the flavonoids' potential as NLRP3 inhibitors. Among the tested flavonoids, all demonstrated favourable interaction profiles with the NLRP3 active site, with quercetin derivatives, particularly Q3-Neo and Manghaslin, exhibiting the strongest inhibitory interactions *in silico* through hydrogen bonding with key amino acid residues. Other flavonoids, including Narcissin and Nicotiflorin, also exhibited significant inhibitory potential, while Clitorin and K3-Neo displayed weaker binding dynamics. The interactions between the hydroxyl groups of the ligands and the carboxylate side chains of Glu629 and Arg578, observed in this study, align with previous findings. These computational findings were corroborated by *in vitro* assays, which demonstrated significant inhibition of NLRP3 inflammasome activation. These findings pave the way for further investigations into their mechanism of action and potential clinical applications.

P42 Antioxidant and antibacterial activities of *Talisia esculenta* leaf extracts

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Talisia esculenta, a native species of northeastern Brazil, produces fruits that are commonly consumed fresh and also incorporated into pastries. In traditional medicine, leaf infusions of *T. esculenta* are used for the treatment of conditions such as hypertension, joint pain, and rheumatism. However, despite its widespread ethnobotanical use, there is still a lack of comprehensive scientific and toxicological data supporting its efficacy. Previous study verified that the aqueous leaf extracts of *T. esculenta* can have a protective effect against oxidative stress in both 3T3 fibroblast cell cultures and zebrafish models [1]. Also, the separation of ethyl acetate and *n*-butanol partitions from water infusion using high-speed countercurrent chromatography (HSCCC) allowed for the isolation of two flavonoids: Quercitrin (quercetin-3-O-rhamnose) and Rutin [2]. This study evaluated the antioxidant activity of *Talisia esculenta* leaf infusion and its ethyl acetate and *n*-butanol partitions. Compared to the infusion, the partitions exhibited two- to three-fold higher antioxidant activity in assays measuring total phenolic content, total antioxidant capacity (TAC), and DPPH radical scavenging. In contrast, copper chelation activity seen for the extracts was approximately 50 % of that observed for the infusion. Antimicrobial activity was assessed for the partitions and selected isolated biomolecules against *Staphylococcus aureus* NCTC 6571, *Escherichia coli* MG1655, *Pseudomonas aeruginosa* PAO1, *Listeria monocytogenes*, and *Salmonella enterica* serovar Typhimurium UK1. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values were observed only for *S. aureus*. Both water infusion and *n*-butanol partition exhibited MIC and MBC values of 12.5 mg/mL. The ethyl acetate partition showed activity at 25 mg/mL. Among the isolated compounds, quercitrin (quercetin-3-O-rhamnose) had MIC and MBC values of 12.5 mg/mL, while rutin demonstrated activity at 17 mg/mL. These findings underscore the potential of *Talisia esculenta* as a source of bioactive compounds with antioxidant and antibacterial properties, particularly against *Staphylococcus aureus*. Further studies are needed to elucidate its mechanisms of action and expand its evaluation to a broader spectrum of microbial pathogens.

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P43 Combinatorial activity of actinomycin D and X2 isolated from *Streptomyces parvus* 35M1 against *Escherichia coli*

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The rise in antibiotic tolerance and resistance among Gram-negative bacteria and the limited development of new antimicrobial agents necessitate alternative treatment approaches such as combination therapy [1]. *Streptomyces* species are well-known natural product factories capable of synthesizing diverse bioactive compounds, including antibiotics, antifungals, and anticancer agents [2,3]. Actinomycin derivatives, produced by *Streptomyces* sp., exhibit weak to moderate activity against Gram-negative pathogens [4]. To enhance their potency, this study aimed to evaluate the synergistic activity of actinomycin D

and X2 with three clinically relevant antibiotics (polymyxin B, kanamycin, and nalidixic acid) against *E. coli* (ATCC 25922). The actinomycin derivatives used were isolated from marine-derived *Streptomyces parvus* 35M1. MIC studies indicated weak to moderate activity (203.91 and 100.83 µM) against *E. coli*. Later, a checkerboard assay was conducted to determine synergy, and inhibition data were analyzed using the Loewe and Bliss independence models via SynergyFinder. Strong synergy was found when the compounds were combined with polymyxin B (1/4 x MIC) and kanamycin (1/2 x MIC), decreasing the MIC value of actinomycins at least four-fold. Scanning electron microscopy revealed significant structural damage on *E. coli*, including abnormal elongation, ruptures, and dent formation. These findings suggest that actinomycin D and X2 can enhance the efficacy of nalidixic acid, polymyxin B, and kanamycin against *E. coli*, highlighting insights into novel strategies for combating Gram-negative bacteria.

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P44 Discovery of anti-inflammatory phytoconstituents from *Lactuca indica* L. based on its traditional uses

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The aerial parts of *Lactuca indica* L. have been traditionally used to relieve skin inflammation or to alleviate stomach-ache. As a continuation of our work, this study aims to evaluate pharmacological activities relating to traditional usage of the plant, with a special focus on anti-inflammatory, anti-bacterial and cytotoxic effects of extracts. The ethanolic extract was prepared by means of percolation, from which 5 sub-extracts were obtained by sequential liquid-liquid partition, including *n*-hexane, chloroform, ethyl acetate, *n*-butanol and aqueous sub-extracts. Through preliminary screening, the *n*-hexane, chloroform and ethyl acetate sub-extracts showed noteworthy inhibition on NO excessive synthesis in LPS-stimulated RAW 264.7 cells. Only the *n*-hexane sub-extract demonstrated anti-bacterial effect. None of the sub-extracts expressed cytotoxic effects on human acute leukemia (HL-60), hepatocellular (Hep-G2) or gastric (MKN-7) carcinoma cell lines. Phytochemical investigations of anti-inflammatory constituents were carried out for *n*-hexane, chloroform and ethyl acetate sub-extracts, affording the isolation of 12 compounds: stearic acid (1), glyceryl monostearate (2), β-amyrin acetate (3), oleanolic acid (4), lupeol (5), 4α(15), 11β(13)-tetrahydroidentin B (6), 5-hydroxymethylfurfural (7), *p*-hydroxymethylbenzoic acid (8), protocatechuic acid (9), succinic acid (10), cynaroside (11) and lynarin (12). Only compound (6) did not exhibit the inhibitory activity on NO synthesis and showed cytotoxicity on RAW 264.7 cells. Other compounds possessed remarkable activity with IC₅₀ values from 1.99 to 39.81 (µM). This study suggests that *L. indica* may be the source of promising

phytochemicals against inflammation mediated by excessive production of NO. This could be a mechanism contributing to partly explain for traditional applications of this herb [1–5].

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P45 Phytochemical analysis, antioxidant and antimicrobial properties of *Cyclamen cypricum*, the national plant of Cyprus

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Cyclamen cypricum is a species in the family Primulaceae, native to Cyprus, where it grows in the wild, particularly in rocky and shaded areas. This species is notable for its beautiful, upswept flowers and attractive foliage [1]. Even though it is not classified as an aromatic plant, it still produces secondary metabolites, a rich source of bioactive compounds that are important for their potential applications in medicine and pharmacy [2]. The aim of this study was to prepare extracts using different solvents from the aerial parts of *Cyclamen cypricum* and to characterise their chemical content in terms of phenolics and flavonoids using the Folin Ciocalteu and the aluminum chloride methods respectively [3]. The antioxidant activity of the plant was evaluated using the ABTS and the DPPH colorimetric assays [4]. Finally, the antimicrobial activity was tested against bacteria using the MIC/MBC microbroth method [5]. Results revealed that all extracts contained glycosylated flavonoids, terpenoid saponins and anthocyanidins. Comparison of the antioxidant activity of the extracts demonstrated higher scavenging activity in the acetone extract, probably attributed to the higher content of phenolics and flavonoids present. The antimicrobial activity showed a MIC range of 0.15–1.25 mg/ml with a preference to *Enterococcus faecalis* and with the acetone extract exhibiting higher antibacterial activity. In summary, our data present significant differences in the antioxidant and antimicrobial activity of the extracts prepared with different solvents. This study also provides valuable data on the phytochemical composition of *C. cypricum*, thus contributing to a better understanding of its biological activities and health benefits.

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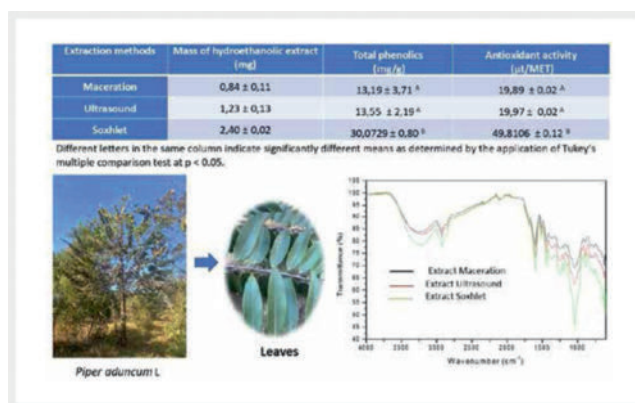
P46 Influence of the extraction method on the phytochemical composition and antioxidant activity of *Piper aduncum* L.

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Piper aduncum L., commonly known as monkey pepper, is widely used in traditional medicine due to its antioxidant and antimicrobial properties. Its leaves contain bioactive compounds with biotechnological potential, whose extraction may vary depending on the method employed. This study analyzed the phytochemical profile of extracts obtained through three techniques: maceration (48 h), ultrasound (1 h), and Soxhlet (4 h), using 70 % ethanol (v/v). The analyses included phytochemical screening via infrared spectroscopy using the attenuated total reflectance method (ATR-FTIR), total phenolic quantification (Folin-Ciocalteu) [2], antioxidant activity evaluation (DPPH) [3], and yield calculation. Leaves of *P. aduncum* were collected in Frutal-MG, Brazil, dried, ground, and subjected to extraction (10 g/125 mL). Among the methods tested, Soxhlet extraction demonstrated the highest yield (2.39 %), followed by ultrasound (1.23 %) and maceration (0.84 %). Soxhlet also stood out in total phenolic content (30.07 mg/g), while maceration and ultrasound showed comparable values (13.19 and 13.55 mg/g, respectively). Antioxidant activity followed a similar trend: Soxhlet achieved the highest value (37.69 μ MET), compared to maceration (19.89 μ MET) and ultrasound (19.97 μ MET). FTIR analysis revealed signals at 3217, 1696, 1595, 1426, 1248, and 1039 cm^{-1} , associated with phenols, flavonoids, and tannins. Weak bands at 2921 and 2842 cm^{-1} suggested triterpenoids and steroids. Notably, phenolic-related signals, especially at 3217 (-OH), 2970 (-CHAr) e 1056 cm^{-1} (C-O), were more intense in the Soxhlet extract, corroborating the quantitative results. Soxhlet extraction proved to be more efficient in recovering these bioactive compounds, offering higher yield and shorter extraction time [1, 2] (► Fig. 1).



► **Fig. 1** Schematic representation of the *Piper aduncum* L. tree, the leaf part used, and the steps for quantifying total phenolics, antioxidant activity, and phytochemical characterization by FTIR.

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P47 *Lagenaria siceraria* var. *longissima* (tenerumi) leaves, a source of N-acylated aromatic amino acids

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Lagenaria siceraria (Molina) Standley is a member of the Cucurbitaceae family, also called Bottle gourd [1]. There are two common types: a short-necked ornamental type, var. a fiasco, and a long edible type, var. *longissima*. The fruit of *longissima* variety is commonly used as a low-calorie vegetable, widely spread in Sicily where it is grown in home gardens and peri-urban farms. The shoots and leaves, called “tenerumi”, are very appreciated in Sicily [2]. The fruit, seeds, leaves, and flowers of *L. siceraria* contain various phytochemicals, such as flavonoids, sterols, saponins, and terpenoids which have been reported to exhibit diverse pharmacological activities including antioxidant, cardioprotective, diuretic, hepatoprotective, hypolipidemic, central nervous system stimulant, anthelmintic, antihypertensive, and analgesic effects [3]. To enhance Italian agri-food products, a phytochemical investigation of *L. siceraria* var. *longissima* leaves was conducted. The ethanol extract obtained by Ultrasound-Assisted Extraction (UAE) was investigated by Liquid Chromatography coupled to High-Resolution Mass Spectrometry (UPLC-Q-Exactive-Orbitrap MS/MS) in negative ion mode. Moreover, a phytochemical investigation allowed us to isolate natural compounds belonging to the class of flavonoids, phenolic acids, glycosylated phenolic acids, caffeic acid derivatives, and N-acylated aromatic amino acids. Their structures were assigned by mono- and bi-dimensional NMR and mass spectrometry experiments. N-acylated aromatic amino acids, also known as lipoamino acids, are a subclass of fatty acid amides which are receiving growing attention due to their structural similarity to endocannabinoids [4]. These compounds, rarely identified in the plants, are reported for the first time in *L. siceraria* leaves.

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P48 Influence of lavender flowers extract on the composition of human gut microbiota ex vivo

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Lavender, especially *Lavandula officinalis*, is widely used to address mild mood disorders and insomnia. While effects of lavender oil on the nervous system are well-known, the impact of its water extract is less studied. Growing interest in gut microbiota (GM) underscores its role in health, as plant compounds can affect various bodily functions. However, the interaction between GM and the bioavailability of lavender's active compounds remains unclear. This study aimed to investigate the biotransformation of compounds contained in lavender infusion by human GM *ex vivo* [1]. To meet the goal the metabolites formed after the incubation with bacteria were examined. Additionally, changes in biodiversity of the GM were observed. The bacterial orders identified included *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria*. GM varies across the gastrointestinal tract and can change within individuals due to factors like aging, antibiotic use, and diet. Although changes in microbiota biodiversity were not statistically significant, qualitative differences were noted between the extract and control groups, especially in the abundance of certain microbial families (Fig. 1.) [2].

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P49 Metabolic trade-offs between growth and secondary metabolism in *Thymus × citriodorus* under drought and salinity stress

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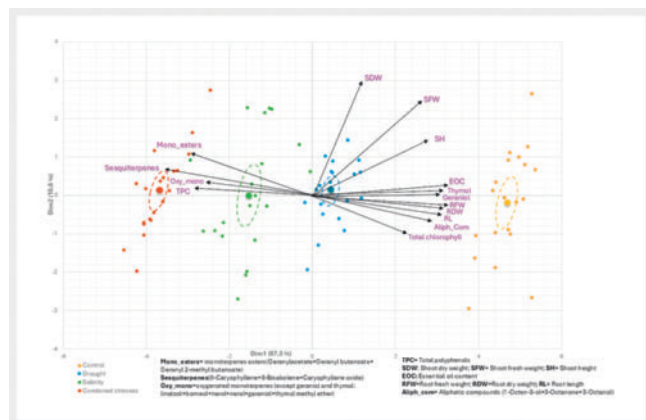
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Medicinal plants often face a trade-off between biomass production and secondary metabolism, particularly under abiotic stress [1]. This study explores whether *Thymus × citriodorus* reallocates resources from growth to the synthesis of bioactive compounds when exposed to drought (40 % SWC), salinity (120 mM NaCl), and their combination.

Morphological and physiological assessments, including shoot and root development, chlorophyll content, were conducted alongside biochemical analyses of essential oil yield, composition, and polyphenol content to clarify stress-induced metabolic shifts. The combined stress exhibited the most severe suppression on biomass-related traits, including shoot height, shoot fresh and dry weights, root length, root fresh and dry weights, and total chlorophyll content, followed by salinity stress, with drought having a milder impact.

Although essential oil yield declined under stress, its composition shifted notably, favouring increased monoterpene esters and sesquiterpenes. Key oxygenated monoterpenes, including neral, geranial, and others, increased under stress, whereas thymol, geraniol, and aliphatic compounds declined, indicating a stress-induced metabolic reconfiguration. Polyphenol content exhibited a significant rise under stress, particularly in combined treatments, reflecting an adaptive response to oxidative pressure. A strong negative correlation between biomass accumulation and secondary metabolite production reinforces the presence of a metabolic trade-off as shown in ► Fig. 1.



► **Fig. 1** Biplot of principal component analysis (PCA) illustrating the relationships among the studied parameters of *T. citriondorus* when subjected to various stress conditions. Dim = dimension.

T. citriondorus prioritizes secondary metabolism overgrowth when challenged by environmental stress, particularly by altering essential oil composition [2, 3]. These findings provide valuable insights into stress-driven metabolic adjustments, offering strategic approaches for optimising bioactive compound production in medicinal plant cultivation.

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P50 Fungal seed-borne endophytes metabolites as sustainable enhancers of tomato seed germination under stress conditions

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Tomato (*Solanum lycopersicum*) is one of the most important crops worldwide and tends to be cultivated in regions with harsh climatic conditions due to its widespread demand. Seed germination represents a critical phase in plant development, directly impacting crop establishment and yield. However, climate change exposes seeds to challenging environments, including emerging phytopathogens and increasing abiotic stresses such as salinity and drought.

To address these challenges and promote sustainable alternatives to synthetic treatments, we investigate the beneficial impact of fungal seed-borne endophytes in enhancing plant fitness. Despite their ecological importance, these microorganisms remain underexplored. Yet they are known to produce a wide range of bioactive metabolites that may contribute to improve stress tolerance [1].

Our objective is to identify and exploit fungal metabolites that enhance tomato seed germination under stress conditions. To this end, we isolated and identified 63 cultivable fungal strains from a worldwide tomato seed collection.

Chemical extracts from these strains were screened for bioactivity against phytopathogens and evaluated for their ability to promote germination under saline and drought conditions. Furthermore, LC-MS/MS analysis combined with molecular networking was performed to dereplicate the metabolomes of the most promising strains.

This approach led to the annotation of known bioactive compounds in one of the most active extracts. Additionally, purification efforts are ongoing to discover novel high-value molecules that could serve as environmentally friendly alternatives to conventional pesticides.

This research highlights the potential of fungal seed-borne endophytes as a source of sustainable biostimulants, supporting the development of more resilient agricultural practices.

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P51 Polyphenol-rich extracts of African *Combretum* and *Terminalia* species show growth inhibition against *Salmonella enterica* and *Bacillus cereus*

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Species of the genera *Terminalia* and *Combretum* occur in savanna woodlands in East, West and South Africa and are used in traditional medicine for various infections including food poisonings [1–3]. Accordingly, this study aimed to assess the effects against *Salmonella enterica* and *Bacillus cereus* of extracts made from various plant parts of *Combretum* and *Terminalia* spp. collected from Tanzania. In addition, we assessed the polyphenol composition of the active plant extracts, and identified the possible specialized metabolites causing the activities. Our results indicated that methanol and chloroform extracts of the leaves of *C. adenogonium* (MIC 156 and 78 µg/ml), and methanol extracts of *T. kaiserana* and *C. collinum* (MIC 313 µg/ml for both) gave the strongest effects against *B. cereus*. Besides, the methanolic root extract of *T. kaiserana* and the leaf decoction of *C. adenogonium* were active against *B. cereus* (MIC 1250 and 2500 µg/ml). Only the methanol extract of the rootbark of *C. zeyheri* showed activity against *S. enterica* (MIC > 2500 µg/ml) whereas all other screened extracts lacked the activity against this bacterium. Our UPLC/HRMS results (ESI-mode) indicated that a methanol extract of *T. kaiserana* roots contained e.g. punicalin (m/z 781.0591), castalagin (m/z 933.0136), ellagic acid rhamnoside (m/z 447.0510), methyl-flavogallate (m/z 483.0253) and punicalagin (m/z 1083.0574), while a gallotannin (m/z 647.0890) was the most abundant metabolite in *C. zeyheri* roots. Our metabolite screening results indicate that *Combretum* and *Terminalia* spp. contain active polyphenolic compounds that could be used for the prevention of food poisonings.

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P52 Tincture of *Aralia mandshurica* root: potential therapeutic applications in chronic disease

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Aralia elata var. *mandshurica* (Rupr. & Maxim.) J. Wen, known as Manchurian aralia (devil's tree), is a plant belonging to the *Araliaceae* family. It naturally occurs in the northeastern regions of Asia, mainly in China, Korea, and Russia. It contains a variety of biologically active compounds, such as saponins, flavonoids, and polysaccharides, which contribute to its medicinal properties. This plant exhibits adaptogenic effects, supporting the body in combating stress and fatigue. In traditional medicine, it is used to enhance immunity and improve nervous system function. Due to its rich phytochemical composition, the root of *A. mandshurica* may serve as a valuable source of compounds used in the prevention of chronic diseases [1]. The main aim of the study was to evaluate the biological properties and chemical composition of a tincture prepared from the root of *Aralia mandshurica*. The study demonstrated that the tincture of *A. elata* var. *mandshurica* possesses strong antioxidant properties, effectively scavenging free radicals and reducing Fe^{3+} and Cu^{2+} ions. The extract inhibited lipid peroxidation and showed significant activity against key enzymes involved in inflammation, neurodegeneration, and metabolic disorders. Phytochemical analysis confirmed the presence of biologically active compounds, such as saponins (aralosides and elatosides) and flavonoids, reinforcing its medicinal potential.

The study demonstrated that tincture of the root exhibits strong antioxidant and enzymatic inhibitory properties, reinforcing its potential therapeutic applications and supporting its traditional use in medicine.

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P53 Phytochemical composition and biological properties of selected *Scutellaria* species

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Scutellaria species, belonging to the *Lamiaceae* family, are rich in flavonoids such as baicalein, baicalin, wogonin, and wogonoside. The most well-known species, *Scutellaria baicalensis* (SB), has been used in traditional Chinese medicine to treat asthma, allergies, inflammatory gastrointestinal conditions, and diarrhea. It exhibits antifungal and antiviral properties, adaptogenic effects, and potential in combating COVID-19 [1]. Other species, such as *S. barbata* (SBa), known for its anticancer properties, and *S. lateriflora* (SL), used for its anti-anxiety effects, have diverse medicinal applications. In Poland, *S. galericulata* (SG) was traditionally used to treat digestive disorders [2]. The main aim of the study was to compare the composition and biological activity of the aerial parts of selected *Scutellaria* species cultivated under Polish climatic conditions.

Studies on selected *Scutellaria* species revealed significant differences in their phytochemical composition and biological activity. SBa had the highest polyphenol and flavonoid content, while SG had the lowest. The strongest antioxidant activity was observed in SBa, as indicated by low IC_{50} values for DPPH and ABTS and high reducing activity. Meanwhile, SB showed the best iron-chelating ability. Additionally, SBa and SB most effectively inhibited the

activity of enzymes such as hyaluronidase, tyrosinase, and acetylcholinesterase, highlighting their potential therapeutic properties.

The diverse phytoactive composition and biological activity of the studied *Scutellaria* species suggest their potential application in the treatment of diseases related to oxidative stress, neurodegeneration, and inflammatory processes, with *S. barbata* and *S. baicalensis* appearing to be the most promising sources of natural enzyme inhibitors and antioxidants.

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P54 Bioactive compounds of *Astragalus* in the prevention and treatment of chronic diseases

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The genus *Astragalus* is one of the largest plant groups in the legume family (*Fabaceae*), comprising over 3,000 species. It has diverse morphology, with plants primarily found in temperate and mountainous regions of Asia, Europe, and North America. Among them is *A. membranaceus*, recognized for its use in traditional medicine, particularly in China. *Astragalus* sp. is valued for its immune-boosting properties and its ability to treat ailments like fatigue and infections. The plant is also the subject of numerous scientific studies confirming its therapeutic potential. Its applications in traditional and modern therapies make it important in natural medicine [1]. This study aimed to compare the chemical composition and biological activity of *Astragalus* species naturally occurring in Poland (*A. cicer* and *A. glycyphyllos*) with *A. membranaceus*.

The root of *A. membranaceus* exhibits the strongest antioxidant and anti-enzymatic properties among the studied plants. On the other hand, the herb *A. cicer* also shows antioxidant and anti-enzymatic activity, though to a lesser extent. Extracts from both plants contained isoflavonoids, primarily formononetin, genistein, and daidzein, which are responsible for these beneficial properties. The anti-enzymatic action of these plants includes inhibiting the activity of enzymes such as hyaluronidase, amylase, glucosidase, and lipoxigenase. The root and herb of *A. glycyphyllos* showed the weakest antioxidant and anti-enzymatic properties.

The research results indicate qualitative similarities and quantitative differences between the analyzed raw materials, leading to varied antioxidant activity. These differences may affect the diversity of biological effects and the potential therapeutic efficacy of the studied raw materials.

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P55 Metabolic characterization of seven bee pollens: Molecular network, metabolite isolation and antioxidant activity assessment

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In a changing world, bees are facing increasing stressful conditions, such as ozone and pollutant concentrations, and more events of higher temperatures [1–3]. Vanderplanck et al., coordinating the BeeMed project, suggested that bees may develop an auto-medication behaviour by selecting the species that may provide them the most beneficial pollen content, notably antioxidant metabolites [4]. The objective of this study, part of the BeeMed project, was to characterize the metabolic content of bee pollens from five dominant species (*Prunus* sp., *Cistus* sp., *Malus* sp., *Salix* sp., *Taraxacum officinale*) and two mix of species (fruit trees, multifloral). A Featured-Based Molecular Network (FBMN) was realized after UHPLC-MS/MS analysis to underscore the occurrence of species metabolites. Major metabolites from the *Prunus*-dominant pollen were purified by Centrifugal Partition and preparative chromatographies for further chemical characterization (1D and 2D NMR). Extracts and purified metabolites were assessed for their antioxidant properties using an online-antioxidant ABTS assay.

Three main spectral families were detected in all bee pollen, namely polyamines (ex: spermidines, spermines), flavonoid glycosides and amino-acids. Specific benzyloquinoline alkaloids (ex: scoulerin and derivatives) were exclusively detected in *Cistus*-dominant pollen.

Isorhamnetin dihexoside, displaying antioxidant properties, and isorhamnetin hexoside were purified from *Prunus*-dominant pollen and structurally confirmed. Myricetin dihexoside and *p*-coumaroyl dicaffeoyl spermidine isomers demonstrated the best antioxidant activities.

The use of three complementary approaches (MS-based FBMN, metabolite purification and anti-oxidant assay) led to a better description of pollen chemical diversity and pave the way for further experiments to better understand bee resilience facing oxidative stresses.

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P56 Evaluation of antioxidant, antimicrobial, and cytotoxic activities of *Digitalis davisiana* Heywood

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The genus *Digitalis* L. (Plantaginaceae), commonly known as foxglove, is a key natural source of cardenolides, with diverse pharmacological properties, particularly in treating cardiovascular diseases [1]. In Türkiye, *Digitalis* is represented by eight species and nine taxa, including *D. lanata* Ehrh. and *D. ferruginea* L., known for their high cardenolide content. *D. davisiana* Heywood, an endemic taxon, remains largely unexplored in terms of its phytochemical and biological properties [1–3].

This study investigates the biological activities of methanol and ethyl acetate extracts from *D. davisiana*. Antioxidant potential was assessed using DPPH radical scavenging activity, where the methanol extract exhibited 43,56% inhibition at 125 µg/mL, while the ethyl acetate extract showed 69,06% inhibition at 1250 µg/mL. The total phenolic content (TPC) was measured using the Folin-Ciocalteu method. The methanol extract exhibited a higher TPC

(60.93 ± 0.05 mg GAE/g) than the ethyl acetate extract (40 ± 0.005 mg GAE/g), suggesting that methanol is more effective in extracting phenolic compounds. Antimicrobial activity was assessed using the microdilution method, revealing that both extracts were active against *Bacillus subtilis* NRRL B478, *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, *Candida albicans* ATCC 90028, and *Candida krusei* ATCC 6258 at MIC values of 1025–625 µg/mL. Cytotoxicity tests (MTT assay) on NIH3T3 cells showed IC₅₀ values above 500 µg/mL.

These findings suggest that *D. davisiana* may be a valuable source of bioactive compounds with antioxidant and antimicrobial potential. Further research is needed to evaluate its pharmacological properties and ensure its safe application fully.

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P57 Uncommon compounds from *Echinophora tournefortii* Jaub. & Spach and their biological activity

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The Apiaceae family, which is home to many of the plants inspired by the production of natural medicines and stands out with its economically significant plants, has approximately 450 genera and 3700 species worldwide. The genus *Echinophora* L., belonging to the Apiaceae family, is represented in the flora of Türkiye with six species, three of which are endemic. *Echinophora tournefortii* Jaub. & Spach, folk known as “spiny Çörtük/Çördük, Tarhana herb” is used as antifungal, antispasmodic, and in stomach ulcers as a wound-healing agent. In addition to phytochemical studies on *Echinophora* species, which are mostly focused on essential oils, there are also studies revealing the presence of alkaloids, flavonoids, polyphenols, phytosterols, polyacetylenes, terpenes, and saponins as secondary metabolites. Their antibacterial, antioxidant, and anti-inflammatory activities are known mostly due to their phenolic components. The phytochemistry of the above-ground parts of this plant has never been investigated so far. This study aims to isolate and elucidate secondary metabolites from *E. tournefortii* and investigate their antioxidant and DNA damage-protective activity. The aqueous MeOH extract of air-dried above-ground parts of *E. tournefortii* was fractionated with *n*-hexane followed by *n*-BuOH. *n*-BuOH fraction was further applied to various column chromatography systems. Isolated compounds were determined as flavonol and flavan glycosides (F1-5), rare 4-hydroxybenzyl alcohol glucosides (HBA1-6), and polyacetylene (P1) by using the spectral (1D and 2D NMR, Mass Spectroscopy, HR-MS) methods. Also, total phenolic content, antioxidant capacity (CUPRAC, FRAP, and TEAC), and DNA damage protective effects of plant extracts (MeOH, *n*-BuOH, hexane, aqueous) and isolated compounds were determined. According to the results, high antioxidant capacity and DNA damage-protective effects were observed. Our phytochemical and different biological activity screening experiments on *E. tournefortii* and other species are ongoing. These plants deserve further investigation as possible drug candidates.

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P58 Evaluation of antioxidant, antimicrobial, and cytotoxic activities of *Teucrium kotschyianum* Poech

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The genus *Teucrium* L. (Lamiaceae) are native to the Mediterranean region and they are known for their antioxidant, antimicrobial, antidiabetic, anti-inflammatory activities [1]. There are 48 species of this genus in Türkiye, 18 of which are endemic [2]. The taxon *Teucrium kotschyianum* Poech remains underexplored and requires further research to fully elucidate its phytochemical and biological properties.

The antioxidant activities were evaluated in ethyl acetate and 70 % ethanol extracts from *T. kotschyianum*. The total phenolic content (TPC) was 64.5 ± 0.07 mg GAE/g for ethyl acetate and 74.4 ± 0.05 mg GAE/g for 70 % ethanol extract. In the DPPH assay, 70 % ethanol extract showed 47.88 % inhibition, while ethyl acetate extract showed 30.35 %, indicating better antioxidant activity in 70 % ethanol. The antimicrobial activity of the extracts was tested by the microdilution method. The antimicrobial MIC values were 1040 µg/mL against *Bacillus subtilis*, *Escherichia coli*, and *Staphylococcus aureus*, 4160 µg/mL against *Candida albicans*, and 2080 µg/mL against *Pseudomonas aeruginosa* and *Candida krusei*. The cytotoxic activity of the extracts was evaluated using the MTT assay and NIH3T3 mouse embryonic fibroblast cells were used for the cytotoxicity test. The IC₅₀ of the ethyl acetate extract (63.4 ± 0.45 µg/mL) lower than the 70 % ethanol extract's (97.3 ± 0.55 µg/mL), suggesting stronger cytotoxicity in the ethyl acetate extract.

These results suggest that *T. kotschyianum* has potential as a source of bioactive compounds with antioxidant, antimicrobial and cytotoxic effects. Further studies are needed to explore its pharmacological properties.

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P59 Antimicrobial potential of *Salvia karwinskii* Benth. exudate against plant pathogens

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Significant losses in the agricultural sector are caused by phytopathogenic fungi and bacteria, which harm food production and cause health problems. While synthetic pesticides offer control, they are associated with environmental concerns and issues of resistance [1]. Given these challenges, we investigated the biological activity and phytochemical composition of dichloromethane surface

extracts from the aerial parts of *Salvia karwinskii*. The exudate yielded several known clerodane-type diterpenoids and exhibited strong antifungal and moderate antibacterial activity. *In vitro* antifungal activity was evaluated against nine phytopathogens and compared to synthetic fungicides [2]. Notably, at a concentration of 1 mg/mL, the extract completely inhibited the mycelial growth of *Phaemoniella chlamydospora*, with efficacy statistically comparable to the synthetic fungicide Score® 25 EC. At 750 µg/mL, it significantly inhibited *Pythium dissotocum* growth by 86.7 %, while *Stemphylium* sp. inhibition reached 94.3 %, a level comparable to Ridomil Gold® WG. The extract showed moderate inhibition against *Fusarium oxysporum* f.sp. *lactucae* race 1 and strong activity against *Alternaria solani*, *Fusarium solani*, *Phoma betae*, and *Colletotrichum lindemuthianum*. Furthermore, at 1000 µg/mL, the extract inhibited *Botrytis cinerea* mycelial growth by 97.2 %, similar to the commercial fungicide Switch®. Its efficacy was also tested against gray mold disease in tomato fruits caused by *B. cinerea*, where curative treatments at just 100 µg/mL provided complete decay control, comparable to peracetic acid. These findings highlight the potential of *S. karwinskii* exudate as a natural alternative for agricultural pest and disease management. Ongoing phytochemical studies aim to identify novel bioactive compounds that could further enhance its applications.

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P60 Combating uropathogenic *E. coli*: from natural products to new molecular targets

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Urinary tract infections (UTI) are one of the world wide infections with immense costs for society. UTI are mostly caused by uropathogenic *E. coli* (UPEC), specifically adapted to the urogenital system by various virulence factors (adhesins, toxin, iron acquisition system, fitness-motility genes, immune evasion). Systematic investigations of traditionally used medicinal plants indicate antivirulent activity under *in vitro* and *in vivo* conditions. Hydroethanolic extracts from *Orthosiphon stamineus* are strong inhibitors of bacterial adhesion to bladder and kidney cells, due to polymethoxylated flavones. Infection studies in mice indicate comparable effects of the extract (750 mg/kg p.o.) to standard norfloxacin treatment. The antivirulent effect is due to inhibition of the bacterial chaperon-usher system, leading to impaired formation of the fimbrial structures. Cranberry fruit extract (*Vaccinium macrocarpon*) induces Tamm-Horsfall Protein (THP) in the kidney as shown within a biomedical study in 16 healthy volunteers [1]. This again leads to inhibition of bacterial adhesion, as THP blocks the mannose-binding side of FimH. Therefore cranberry extract can be assessed as an inductor of innate immune defense. Infusions of *Equisetum arvense* are strong inducers of THP after 8 days of oral intake in humans besides diuretic effects [2]. Malonylated dammaranes from *Betula* sp. interact with the mannose-binding domain of FimH of UPEC, leading to reduced bacterial adhesion and as a counter-reaction to significant increase in bacterial motility. These data support the traditional use of many herbal materials from European Medicine for UTI and synergism indicate that also combinations of herbal materials can have better effects than the individual plants.

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P61 Anti-inflammatory and antitussive efficacy of ivy leaf dry extract EA575 in an acute airway inflammation animal model

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The mode of action of ivy leaf dry extract EA575 (DER 5-7.5:1, extraction solvent 30% ethanol) is well described *in vitro* [1–5] however so far *in vivo* data translating cellular mechanisms into physiological context is scarce. Within two studies, the efficacy to attenuate inflammation in mice and inflammation-associated cough in guinea pigs after twice-daily EA575 treatment (43, 215.25, and 430.5 mg/kg bw/d) for 6 and 7 days, respectively, was elucidated. First, a murine bleomycin-induced acute airway inflammation model was applied to assess anti-inflammatory properties. The study was terminated on day 7, 24 h after the last EA575 treatment and investigated cytology and cytokines in bronchoalveolar lavage fluid (BALF) as well as histopathological changes including goblet cell metaplasia after 3- and 7-days post-bleomycin challenge. EA575 treatment dose-dependently reduced inflammatory cell influx in BALF and lung tissue at day 3 and 7. Furthermore, cytokine levels in BALF, e.g., TNF- α and IL-6, were dose-dependently decreased. Lastly, pathology score of goblet cell metaplasia showed a dose-dependent reduction at both timepoints. Moreover, the impact of EA575 on inflammation-associated cough was investigated in guinea pigs with bleomycin-induced acute airway inflammation after 7 days of EA575 treatment. Hereby, quantification of cough events after citric acid exposure and cytology in BALF were the main endpoints. Upon citric acid exposure, EA575 dose-dependently reduced inflammatory cell influx in BALF and the number of coughing events were accordingly reduced. Together, these observations highlight the potential of EA575 in the treatment of acute airway inflammation, especially during respiratory diseases accompanied with inflammation-associated excessive mucus production and cough.

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P62 Novel steroidal glycosides from the underground parts of *Agapanthus africanus* and their cytotoxicity against human cancer cell lines

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Approximately 25% of all new anticancer agents approved by the Food and Drug Administration between January 1981 and September 2019 were derived from natural products and their derivatives, suggesting that natural products continue to play a crucial role in the development of anticancer agents [1]. As part of our ongoing phytochemical investigations to discover new candidates for anticancer agents from higher plants, we have focused on *Agapanthus africanus*. Previous studies have reported that this plant contains chalcone and dimeric dihydrochalcone derivatives and a steroidal glycoside [2, 3]. However, our literature survey has demonstrated that the chemical constituents of *A. africanus* remain to be fully investigated, prompting us to perform a comprehensive phytochemical investigation of this plant. Sixteen novel steroidal glycosides (**1–16**) were isolated, and their structures were determined primarily by NMR spectroscopic analysis (► Fig. 1). The cytotoxicity of **1–16** against SBC-3 human-small cell lung cancer cells was evaluated, exhibiting IC₅₀ values ranging from 1.2 to 35 μ M. Compound **1**, which showed the most potent cytotoxicity among the isolated compounds, was evaluated for its apoptosis-inducing activity in SBC-3 cells. As a result, **1** arrested the cell cycle at the G2/M phase and induced apoptosis, accompanied by depolarization of mitochondrial membrane potential, overproduction of reactive oxygen species, and a decreased Bcl-2/Bax ratio. These phenomena indicate that **1** induces apoptosis via an intrinsic pathway in SBC-3 cells. Compound **1** represents a promising seed compound for the development of novel anticancer agents targeting small-cell lung cancer.



► Fig. 1 Structures of **1–16**

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P63 A polysaccharide-rich extract of *Astragalus mongholicus* (CPSP-01) as a promising immunomodulatory supplement for managing infections, inflammation, oxidative stress in aging

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Astragalus radix is described for its immunomodulatory, anti-inflammatory and anti-cancer activities. Since aging progressively impairs the innate immune system's ability to mount effective cellular responses against infections, this study aimed to assess the immunomodulatory potential of a polysaccharide-rich aqueous extract of *Astragalus mongholicus* Bunge root (CPSP-01) and its ability to strengthen the anti-infectious immune response in aging.

HPTLC analysis showed that CPSP-01 contains sugars, amino acids, flavonoids and saponosides, and 8 % polysaccharides. By using monocyte-derived macrophages isolated from young or old healthy volunteers, we demonstrated that CPSP-01 strengthens the microbicidal ability of these macrophages to fight against fungal, bacterial and viral infection in the elderly. Consistently, CPSP-01 increased the ability of h-MDMs to produce highly antimicrobial reactive oxygen species, and the expression of C-type lectin receptors and Toll-Like receptors, crucial for pathogen recognition and induction of immunity against these agents. The oral administration of CPSP-01 for 15 days (40 mg/kg once daily) to aged mice showed that the extract promotes the microbicidal activity of macrophages against *C. albicans*, *S. pneumoniae* and Poly(I:C). CPSP-01 dampened inflammation (reduced plasmatic SAP and urinary PGE2 levels) and oxidative status (reduced 8-isoprostane urinary level) in aged mice, through its impact on macrophage differentiation towards an anti-inflammatory and less pro-oxidant phenotype. Altogether, these data highlight the potential of the polysaccharide-rich *A. mongholicus* Bunge extract tested as a promising immunomodulatory supplement to control and prevent infections during aging while alleviating inflammation and oxidative stress.

P64 Immunomodulatory properties of the fresh *Curcuma longa* rhizome extract CPSP-01 on HT-29 cell-line

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Turmeric (*Curcuma longa* L.), widely used as a condiment and flavouring agent, has also long been employed in traditional medicine for its anti-inflammatory and analgesic properties. Most industrial extracts are obtained from heat-treated rhizomes, standardized to high levels of curcuminoids (80-95 %), and largely documented for their biological activities. However, the rhizome of *C. longa* contains a broader chemical diversity, and the biological activity of extracts with lower curcuminoid content but richer phytochemical profiles remain unexplored.

In our study, the properties of the fresh *C. longa* rhizome extract CPSP-01 (25 and 50 µg/mL, 50 % hydroethanolic extract, 8 % curcuminoids, HPTLC-characterized for the presence of terpenes and amino acids) were evaluated on HT-29 cell-line challenged or not with LPS using butyrate as reference. Key markers of immunomodulation, gut barrier function and antioxidative defences were investigated.

Under basal conditions, neither butyrate nor CPSP-01 affected IL-8 secretion or Tnf-α expression. Butyrate upregulated IL-10 without altering Cox-2 expressions. Conversely, CPSP-01 decreased Cox-2 without affecting IL-10 expressions.

Under challenge with LPS, butyrate reduced the pro-inflammatory biomarkers and tended to increase anti-inflammatory biomarkers. CPSP-01 tended to reduce IL-8 secretion, decreased Tnf-α expression at the highest dose, lowered Cox-2 and tended to increase IL-10 expression. Notably, the effect of CPSP-01 on Cox-2 expression was significantly more pronounced compared to butyrate. No significant changes were observed in Zo-1, Occln, Muc-2, or Nrf2 expression, suggesting no effect on the barrier function and antioxidative defences. These findings provide valuable insights into the potential therapeutic applications of CPSP-01 in the modulation of inflammation.

P65 The impact of *Melissa officinalis* L. leaf extract CPSP-02 on gastrointestinal motility: Segmentspecific responses in a mouse model

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Gastrointestinal motility disorders are found in a wide range of pathologies (spasms, stress, type 2 diabetes, irritable bowel syndrome, etc.). *Melissa officinalis* L. (lemon balm) has been traditionally used for decades with symptomatic benefits in patients with digestive disorders and could be a good candidate to reduce hyper-motility. However, the few studies carried out on this plant gave heterogeneous and contradictory results, depending mainly on the type of extracts tested and the gastrointestinal samples used. In our study, the basal and spasmolytic properties of an hydroethanolic extract of lemon balm leaves (CPSP-02; LC-MS characterized) were evaluated, in comparison with a vehicle, *in vivo* (2 days of oral administration; 1.14 mg/mice) and *ex vivo* (3 doses, 0.75, 2 and 4 mg/mL) on different parts of the GI tract from stomach to distal colon. CPSP-02 had no impact on metabolic parameters (body weight, food and water intake). No significant difference was observed in basal conditions between the two groups, vehicle and CPSP-02. However, in acetylcholine pre-contracted segments, the amplitude of contractions was significantly and specifically decreased in the upper digestive segments (stomach, duodenum and jejunum) with CPSP-02 both *in vivo* and *ex vivo*. A clear dose-dependent effect of CPSP-02 was also observed, where different concentrations exerted varying levels of impact across the GI segments. This study highlights the powerful antispasmodic effects of lemon balm CPSP-02 extract, with dose-dependent responses and GI segment-specific effects. The more precise mechanisms of action and the mediators involved remain to be determined.

P66 Telomere-enhancing potential of Limonium species: A novel approach to skin aging and cosmetics

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The genus *Limonium* (Plumbaginaceae) comprises approximately 600 perennial species distributed worldwide, predominantly along Mediterranean coastal regions, as well as in North America, Europe, and Australia. Due to their lavender-like floral appearance, *Limonium* species are commonly referred to as sea-lavender or marsh-rosemary. In South Korea, only one species, *Limonium tetragonum*, is known to be native. This plant is classified as a halophyte, exhibiting high salt tolerance and possessing a robust antioxidant defense system along with various bioactive secondary metabolites.

Telomeres are repetitive DNA sequences located at the ends of chromosomes, playing a crucial role in maintaining chromosomal stability and preventing the loss of genetic information. With each cell division, telomeres gradually shorten, a process directly associated with cellular aging. Telomerase, an enzyme that counteracts telomere shortening, is known to be highly active in stem cells and cancer cells but exhibits low activity in somatic cells. Consequently, telomerase has emerged as a key target in both cancer and aging research.

Recent studies have reported that natural compounds may regulate telomerase activity or contribute to telomere length maintenance. Notably, cycloastragenol and astragaloside IV, derived from *Astragalus membranaceus* root, have been identified as telomerase activators, alongside other bioactive compounds such as resveratrol, curcumin, and quercetin.

In this study, we conducted a comparative evaluation of telomere length extension activity in various *Limonium* species, aiming to identify potential candidates for anti-aging and cosmeceutical applications. Extracts from 11 *Limonium* species, including *Limonium tetragonum*, were assessed for their effects on telomere length in HaCaT cells. Additionally, we examined the mRNA expression levels of hTERT and c-MYC, key regulators of telomerase activity. Our findings revealed a promising candidate with telomere extension activity exceeding that of quercetin and curcumin by more than twofold, highlighting its potential for anti-aging applications.

P68 *Salvia verticillata* L. (Lamiaceae) root extract in PCOS treatment: Phytochemical composition and *in vivo* insights

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The *Salvia verticillata* L. (Lamiaceae) roots have been traditionally used in Serbia to treat various gynecological disorders, including cysts, myoma, and hormone disbalance. *S. verticillata* roots have not been sufficiently studied so far, unlike the aerial parts [1, 2]. Polycystic ovary syndrome (PCOS) is one of the most prevalent endocrine disorders among reproductive-age women and closely associated with metabolic dysfunction. Currently, there is no curative treatment that effectively resolves all symptoms related to PCOS. This study evaluates the potential effects of *S. verticillata* root methanolic extract (SVR) on estrous cycle regulation and glucose metabolism in a rat model of PCOS. PCOS was induced through the simultaneous administration of letrozole (1 mg/kg) and a high-fat diet over a 21-day period. Following successful induction, SVR (200 mg/kg) or metformin (500 mg/kg) was administered orally for 28 days. Estrous cycles were monitored during the final 12 days of the treatment protocol. An oral glucose tolerance test (OGTT) was conducted using 2 g/kg of glucose, with glycemia measured at baseline and after 60 and 120 min. Our results indicate that SVR significantly improved estrous cyclicity in PCOS rats, comparable to the metformin effects. Glycemia levels were notably elevated in the PCOS group compared to both the metformin and SVR-treated groups at all measurement

points. The HPLC-DAD-MS analysis showed the presence of various phenolic compounds, in particular rosmarinic acid. These findings strongly suggest that *S. verticillata* root extract may offer a promising phytotherapeutic approach for addressing PCOS-related symptoms and warrant further investigation.

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P69 Revisiting curcumin, an old ‘friend’ in the era of antimicrobial resistance

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With antimicrobial resistance (AMR) an ever-present spectre and the prospect of 10 million deaths globally due to drug resistance by 2050 [1], scientists from various disciplines continue looking for alternate compounds to treat infections resistant to existing drugs. This includes re-evaluating natural products from plant extracts plus synthesising compounds based upon plant scaffolds. One such candidate is curcumin (Fig. 1), extracted from turmeric (*Curcuma longa*). With a long pedigree of historical medicinal use, from an activity perspective, one of the first published papers described curcumin's antibacterial activity against *Staphylococcus aureus*, *Salmonella paratyphi* and *Mycobacterium tuberculosis*, although much subsequent research has concentrated on anticancer and anti-inflammatory properties due to issues with poor solubility and bio-availability [2]. Although curcumin shows promise against some difficult to treat bacterial species including MRSA and *Clostridium difficile* [3], curcumin derivatives have been synthesised with the aim to improve bioactivity. Curcuminoids synthesised and chemically characterised at Kingston University show minimum inhibitory concentrations between 5 and 20 µg/mL against staphylococci [4], close to that seen with vancomycin (4 mg/L) [5], with one derivative inhibiting biofilm formation at 40 µg/mL. Biofilms, bacterial communities attached to biotic and abiotic surfaces, are associated with numerous bacterial infections and provide a natural defence to bacterial cells within them, making them hard to remove. Such results support continued research on curcumin and its derivatives to treat bacteria of clinical importance.

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P70 Molecular networking-guided isolation of compounds from *Keetia venosa* (Oliv.) Hiern leaves and their antibacterial activities

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The global incidence of antibacterial resistance represents a significant public health challenge, with an estimated number of 4.95 million deaths in 2019, predominantly in developing countries [1]. This emphasises the urgent necessity to identify novel molecules with antibacterial properties to address this concern. Medicinal plants have been identified as playing a crucial role due to their rich chemical diversity and bioactivity [2]. Nonetheless, the conventional approach to natural product isolation remains challenging, as evidenced by the technical limitations associated with in-depth screening of biomaterials. In this study, we have employed a molecular networking-guided approach aiming at the in-depth chemical profiling and isolation of new potential antibacterial compounds from a medicinal plant *Keetia venosa* leaves. These plants are used in traditional medicine to treat bacterial and parasitic infections.

This applied method led to the isolation and characterisation of 18 compounds, including 4 previously undescribed. The structures of these compounds were elucidated through a comprehensive analysis of their physical, HR-MS/MS and NMR spectroscopic data. Several additional compounds were putatively identified by comparing their MS² features with analogous structures in established databases. Moreover, the antibacterial activity of extracts, fractions and isolated compounds were evaluated against a range of Gram-positive and -negative bacteria. Some fractions and isolated compounds displayed promising antibacterial activity against Gram-negative bacteria such as *K. pneumoniae*, *S. enterica* and *P. aeruginosa* with MIC values ranging from 3.9–62.5 µg/mL. The molecular networking-guided applied methodology speeds up the dereplication, targeting and isolation of natural products from plant extracts.

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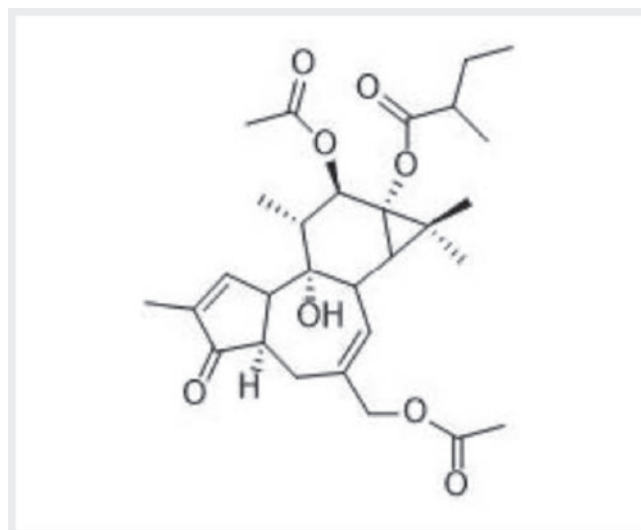
P71 Phorbol esters from *Croton tiglium* leaves and their antibacterial and cytotoxicity activities

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Croton is a genus of the family Euphorbiaceae, known to have around 1300 species worldwide. Several species have a long history in traditional medicine. In the ayurvedic system of medicine, the *Croton tiglium* seed oil is used to ease various illnesses, including obstinate constipation, intestinal obstruction, lead poisoning, and high blood pressure [1]. *Croton tiglium* contains phorbol esters, which have a wide range of biological activities, such as cytotoxicity, anti-inflammatory, and antitubercular effects [2]. The present study aims to evaluate the antibacterial activity of *C. tiglium* leaf extracts, identify bioactive phyto-

chemical compounds, and assess their anticancer potential against MDA-MB 231 breast cancer cells. The hexane extract, which mainly contains hydrocarbons demonstrated the highest antibacterial activity against *Staphylococcus aureus*, with a Minimum Inhibitory Concentration of 12.5 µg/mL and Minimum Bactericidal Concentration of 25 µg/mL. Four novel and eight known phorbol esters were isolated using chromatographic techniques, including gravity column chromatography, autopurification system, and semi-preparative HPLC. Their structures were elucidated using spectroscopic methods. These compounds were screened for cytotoxicity against MDA-MB 231 cells. While no compounds exhibited significant activity, 20-acetyl-12-O-acetyl-4α-deoxyphorbol-13-(2-methyl)butyrate reduced cell viability to 67.8 % (► Fig. 1).



► Fig. 1 20-acetyl-12-O-acetyl-4α-deoxyphorbol-13-(2-methyl)butyrate

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P72 Phytochemical investigation of *Sambucus nigra* L. flowers: Optimization of extraction techniques and comprehensive metabolite profiling

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Sambucus nigra L. (black elder) flowers are widely recognized for their traditional medicinal use. They represent a rich source of bioactive compounds, particularly flavonoids and phenolic acids [1]. The aim of this study was to optimize extraction conditions in relation to the phytochemical composition of *S. nigra* flower extracts, using a wide array of modern analytical techniques. Three extraction methods – ultrasound-assisted extraction (UAE), conventional maceration (shaking), and accelerated solvent extraction (ASE) were tested with varying solvents (water, ethanol, hydroalcoholic mixture), temperatures, and

durations. The resulting extracts were subjected to high-performance liquid chromatography (HPLC) for quantification of major phenolics, and LC-MS for detailed metabolite profiling and tentative identification.

Centrifugal partition chromatography (CPC) was employed to fractionate the optimized extract and deliver fractions enriched in cosmetically significant metabolites. The fractions obtained were chemically profiled using a NMR-based dereplication approach completed with high resolution LC/MS.

Among the tested conditions, ethanol was identified as the most effective solvent, providing the highest yield of phenolic compounds. The accelerated solvent extraction (ASE) was found to be the most efficient extraction technique, providing excellent recovery of bioactive components. Well-chosen CPC fractionation conditions significantly enhanced compound separation, while MS and NMR provided essential structural insights, allowing precise characterization of the bioactive constituents. Together, these methods offer a powerful and comprehensive platform for the study and development of standardized elderflower-based products with potential cosmetic value.

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P73 Changes in the content of active compounds and antioxidant activity of willow bark and leaves collected at different harvest times

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The *Salix* genus is known for its rich species diversity and high phenolic compound content, the amount of which depends on many factors. The conducted study focused on the phytochemical analysis and antioxidant activity of five willow species (*S. cinerea*, *S. fragilis*, *S. purpurea*, *S. triandra*, and *S. viminalis*) growing in natural habitats in Poland. The aim was to determine how the content of active compounds changes, depending on species, plant part, and harvest time. The total phenolic content in the bark increased from April to August in four species before decreasing in October. Notably, in the bark of *S. purpurea*, the total flavonoid content showed the most significant variation. It increased sharply from spring to summer, reaching almost twice the spring level by August, before dropping in autumn to a level similar to that of spring. *S. purpurea* is also the richest in salicin, while the bark of *S. fragilis* contains a high level of (+)-catechin. In turn, chlorogenic and neochlorogenic acids are found at the highest concentration in the leaves of *S. fragilis*. In the leaves, the total flavonoid content was the highest at each harvest time in *S. triandra*. The most significant increase in the total polyphenol and phenolic acid content was observed in autumn in leaves of *S. purpurea*. All studied willow species and materials displayed high antioxidant potential. The study demonstrated that the species, type of raw material, and harvest time significantly influence raw material quality.

P74 Development of novel transdermal therapy for rheumatoid arthritis from *Adenanthera microsperma* Teijsm. & Binn

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Rheumatoid arthritis (RA) is a chronic autoimmune disorder characterized by persistent joint inflammation, pain, and progressive cartilage destruction. Current treatments have limitations, necessitating alternative therapeutic strategies. This study evaluated the anti-arthritis potential of *Adenanthera microsperma*, a species with traditional use in inflammatory conditions [1]. The *in vitro* anti-inflammatory potential of the methanolic extract was evaluated in RAW 264.7 macrophages, where it exhibited significant nitric oxide inhibition. Additionally, the extract demonstrated activity in the *in vitro* anti-arthritis assay using the protein denaturation method. Based on these findings, *in vivo* studies were performed in female Sprague-Dawley rats with CFA-induced arthritis [2]. The extract was administered orally at doses of 200 and 400 mg/kg, demonstrating promising anti-arthritis effects. To enhance site-specific delivery and minimize systemic toxicity, a transferosome-based transdermal gel was formulated [3] and evaluated at 25 mg/kg and 50 mg/kg, with methotrexate gel as the standard. The formulation significantly reduced inflammatory markers: IL-6 (581.03 pg/mL in disease control vs. 219.11 pg/mL at 50 mg/kg), IL-1 β (267.97 pg/mL vs. 97.73 pg/mL), and TNF- α (54.77 pg/mL vs. 24.33 pg/mL). X-ray analysis of the affected ankle joint confirmed reduced bone erosion, and histopathology revealed decreased synovial hyperplasia and inflammatory infiltration. Furthermore, oxidative stress markers (SOD, catalase) were significantly restored. The transferosomal gel formulation effectively alleviated arthritis symptoms, offering a targeted, non-invasive therapeutic approach with reduced systemic side effects. These findings establish *A. microsperma* as a promising natural anti-arthritis agent, warranting further clinical investigation.

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P75 Polyphenol metabolism in the ageing gut: Comparative analysis of olive polyphenol biotransformation between healthy young and elderly

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Life expectancy has never been higher and is predicted to keep rising, shifting the demographic profile to an older population. Ageing is often accompanied by comorbidities and polypharmacy, thereby increasing healthcare system demands [1]. A healthy diet is crucial for longevity and disease prevention, and research into age-related changes in the gastrointestinal tract and the biotransformation of bioactive compounds is becoming increasingly relevant [2].

This research aims to adapt an in-house validated *in vitro*.

Literature was reviewed for changes in transit time, pH, enzyme activity, and gut microbiome composition during ageing [3, 4].

Biotransformation experiments with olive leaf extract resulted in various metabolites due to deglycosylation, hydrolysis, (de)methylation, and flavonoid

ring cleavage. Both age groups formed the same metabolites, but the elderly showed a higher biotransformation rate, possibly due to lower viable cell count in the younger population's faecal pool [5].

In conclusion, the experiments indicate that polyphenols maintain their potential health benefits in healthy elderly individuals since similar biotransformation patterns were observed. Further research on the ageing gastrointestinal tract is essential for advancing research in healthy ageing.

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P76 Evaluation of skin toxicity and irritation effects of *Xanthii fructus* extract using animal alternative models

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Xanthii Fructus (XF), the fruit of *Xanthium strumarium* L., has been traditionally used in Asian medicine for its antibacterial and anti-inflammatory properties, attributed to its bioactive components such as glycosides, phytosterols, and phenolic acids. Recently, XF has gained attention as a promising candidate for dermatological and cosmetic applications.

This study evaluated the cosmetic potential of XF as a cosmetic ingredient by investigating its wound healing effects and skin sensitization in HaCaT cells. Additionally, the reconstructed human epidermis (RHE) model was employed for skin irritation testing, following OECD guidelines.

In the RHE model, XF showed no-cytotoxicity at any tested concentration and reduced the expression of interleukin (IL)-1 β . In TNF- α /IFN- γ -stimulated HaCaT cells, XF treatment also significantly decreased IL-6 levels, indicating its anti-inflammatory potential through downregulation of inflammatory markers IL-6 and IL-1 β . Skin sensitization potential was further evaluated using the Direct Peptide Reactivity Assay (DPRA), where XF showed minimal reactivity to both cysteine and lysine peptides, classifying XF as a Non-sensitizer. A network-based *in silico* analysis revealed distinct gene associations between XF's bioactive compounds, irritation, and wound healing pathways. Among the top ten core genes revealed to wound healing such as TP53, JUN, TNF, and IL6, most were also identified in the irritation-related gene network, with minor differences depending on geographical origin. Notably, both XF extracts were consistently linked to immune response genes including JUN, TNF, and IL-6. In conclusion, XF exhibits anti-inflammatory, non-irritant, and wound healing properties, supporting its potential as a bioactive ingredient for therapeutic skin agent and cosmetic formulations [1, 2].

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P77 Study on the anti-inflammatory and skin regenerative mechanisms of *Xanthium strumarium* L. fruit via modulation of atopic signaling pathways

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Xanthium strumarium (XSE) is rich in bioactive compounds and has been traditionally used for the treatment of inflammation, pruritus, and ulcerative skin conditions. However, despite its traditional use, the molecular mechanisms underlying its therapeutic effects in inflammatory skin diseases remain poorly understood. This study aimed to evaluate the therapeutic effects of XSE on atopic dermatitis (AD) and to elucidate its underlying molecular mechanisms [1–3].

An inflammatory environment was induced in HaCaT human keratinocyte cells using tumor necrosis factor-alpha (TNF- α) and interferon-gamma (IFN- γ). XSE treatment effectively alleviated inflammatory responses in HaCaT cells by suppressing NF- κ B activation, leading to reduced phosphorylation of p38 MAPK and I κ B α . In addition, XSE inhibited activation of both p38 and STAT1 signaling pathways, resulting in downregulation of mRNA expression levels of the inflammatory chemokines TARC and MDC, which are key mediators in AD pathogenesis. mRNA levels were assessed using quantitative real-time PCR (qRT-PCR), and protein expression levels were evaluated by Western blot analysis. Furthermore, XSE promoted nuclear translocation of Nrf2 and activated the Nrf2 signaling pathway via upstream modulation of the PI3K/Akt pathway. This activation subsequently increased the expression of HO-1, an antioxidant enzyme with anti-inflammatory properties. XSE also restored the expression of filaggrin, a key structural protein involved in maintaining the skin barrier, which had been suppressed under inflammatory conditions.

In conclusion, this study demonstrated that XSE ameliorates atopic dermatitis by suppressing the NF- κ B, STAT1, and p38 MAPK signaling pathways while activating the Nrf2/PI3K/Akt axis. These findings highlight the key molecular mechanisms underlying XSE's anti-inflammatory and skin barrier-regenerative effects, supporting its potential as a therapeutic agent for inflammatory skin diseases.

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P78 *Carya illinoensis* nut shell extract, a very valuable cosmetic ingredient

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Carya illinoensis (pecan) nuts are widely used in the food industry. More than 60 thousand tons of shelled pecan nuts were sold in the United States alone in 2023 (nass.usda.gov). The shells represent 40% to 50% of the pecan weight [1] and are considered as wastes. However, pecan shells are known to be rich in polyphenols and tannins [1, 2] but at this time only some low-added-value valorizations have been described. For example, they can be used for bioenergy production [3] or to remove pollutants from water [4]. Very few studies have been conducted for high-added-value applications, such as cosmetics, although anti-oxidant, antibacterial, and antifungal properties have been previously reported from pecan shells for medical applications [2].

Based on these properties, we decided to develop an extract rich in tannins that could be used in cosmetic products. We then investigated various cosmetic

properties of this shell extract and we demonstrated that this extract exhibited many properties of value for cosmetics. Among them, it showed good antioxidant activities on keratinocytes and we also highlighted for the first time anti-ageing properties for *Carya illinoensis* shell extract, thanks to cellular assays on fibroblasts.

Therefore, *Carya illinoensis* shell extract can be used as a very valuable cosmetic ingredient.

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P79 Evaluation of the antioxidant and anti-inflammatory activity of ethanolic extract of leaves of *Baccharis alnifolia* Meyen & Walp

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Traditional uses of *Baccharis alnifolia*, family Asteraceae in the Andean communities of South America include bone pain, indigestion, anti-inflammatory and antirheumatic [1]. The species is important in phytoecology.

Flavonoids comprise a group of polyphenolic compounds widely distributed in plants; they possess antioxidant, anti-inflammatory, anti-thrombotic, anti-microbial, anti-allergic, anti-tumor and anti-asthmatic properties [3].

The study evaluated the anti-inflammatory activity by cell lysis, protein denaturation and antioxidant activity with 1,1-diphenyl-2-picrylhydrazyl (DPPH), of the ethanol extract of the plant with ethanol: 25, 50, 75 and 96 % and temperature: 30, 40, 50 and 60 °C. The highest concentration of flavonoids is at 50 % and 50 °C and 96 % at 30 °C; by UV-Vis spectroscopy; 59.36 mg quercetin/g [2] and 88.32 mg luteolin/g [4]; the quantification of phenols by Folin-Ciocalteu, 140.86 mg GAE/g of extract expressed as gallic acid.

Antioxidant activity, in terms of IC₅₀ of 11.23 ppm. The anti-inflammatory activity by cell lysis of the extract at a concentration of 50 µg/mL at 96 % and 30 °C is 98.63 % protection, superior to the diclofenac standard of 91.43 %; the anti-inflammatory activity by protein denaturation at 100 µg/mL at 50 % and 50 °C, is 40.26 % protection, compared to diclofenac of 35.49 %. The results show the efficacy and safety of the vegetable under study, due to the presence of bioactive components.

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P80 Exploring potential analgesic indole alkaloids from *Picralima nitida* (Akuamma)

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The genus *Picralima* has been traditionally used in African ethnomedicine for its analgesic, antipyretic, and antimalarial properties [1], alongside other plants of the Apocynaceae family. *Picralima nitida* is rich in indole monoterpene alkaloids, some of which interact with opioid receptors [2], as well as serpentine-related bisindole alkaloids [3]. Recently, extracts or ground seeds from *P. nitida* have appeared on the market as substitutes for *Mitragyna speciosa* (kratom). This study aimed to isolate and structurally characterize indole monoterpene alkaloids and serpentine-related alkaloids from *P. nitida* ground seeds using chromatographic techniques, HPLC-HRMS, NMR, and X-ray crystallography. Major alkaloid akuammine was identified alongside several minor alkaloids. Their opioid receptor activity is assessed using Bioluminescence Resonance Energy Transfer (BRET)-based assays, enabling real-time monitoring of receptor activation and biased agonism. Special attention is given to distinguishing between G-protein-biased agonists and β -arrestin recruitment activators, as the latter is often associated with opioid-induced adverse effects. By investigating the structure-activity relationships of these alkaloids, this research aims to provide deeper insights into their potential as natural analgesics. The study includes the identification of opioid-active alkaloids that could serve as leads for the development of safer, plant-derived pain therapeutics. There are no conflicts of interest.

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P81 Agitated *in vitro* cultures of *Scutellaria brevibracteata* subsp. *subvelutina* (Rech.f.) Greuter & Burdet – polyphenolic metabolites production optimisation

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Scutellaria brevibracteata subsp. *subvelutina* is one of the lesser-known plants of the skullcap genus. Occurs in rocky habitats in the region of Palestine, Syria, Lebanon, Turkey, and Saudi Arabia. Based on the results of earlier studies on the chemical composition of other *in vitro* cultures of *Scutellaria* species, it was

recognised that they are a promising research object and, like other species, can be a source of metabolites with medicinal applications [1].

This study aimed to assess the usefulness of agitated cultures of *S. brevibracteata* subsp. *subvelutina* for the production of secondary metabolites. Shoot cultures were maintained for four weeks on Murashige and Skoog medium supplemented with various concentrations of 6-benzylaminopurine and 1-naphthylacetic acid. Qualitative and quantitative analysis of the secondary metabolites content in the biomass extracts was performed by DAD-HPLC.

The presence of six phenolic acids, two phenylpropanoid glycosides, and eight flavonoids was confirmed in the tested extracts. The quantitatively dominant metabolites were ferulic acid, verbascoside, wogonoside, and scutellarin. The highest total content of phenolic acids (109.85 mg/100 g DW), phenylpropanoid glycosides (721.04 mg/100 g DW), and flavonoids (681.80 mg/100 g DW) was obtained, 7.1, 1.6, and 1.2 times higher than in agar cultures, respectively. Most importantly, the biomass increase of agitated cultures was 4.6 times higher than in agar cultures [2].

The evaluation of the obtained results allows us to conclude that agitated *in vitro* shoot cultures of the tested species are a valuable source of flavonoids and verbascoside.

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P82 Bioreactor-grown suspension cultures of *Schisandra henryi*: Polyphenol profiling and anticancer potential of biomass extracts

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Polyphenolic compounds derived from plant extracts are widely recognized for their significant biological activities, including anticancer, anti-inflammatory, and antioxidant effects. *In vitro* plant cultures represent a promising alternative source of polyphenols, with bioreactor-based cultivation offering enhanced secondary metabolite production. This study aimed to establish suspension cultures of *Schisandra henryi* C.B. Clarke in a stirred-tank bioreactor, perform phytochemical profiling of polyphenolic compounds, and evaluate their anticancer potential.

Suspension cultures were initiated and maintained in Murashige and Skoog liquid medium supplemented with 2 mg/L indole-3-butyric acid and 1 mg/L 6-benzyladenine. Biomass was harvested at six time points (days 5, 10, 15, 20, 25, and 30; n = 4), followed by methanol extraction. Polyphenolic compounds were identified via UHPLC-DAD-ESI-MS³ and quantified using HPLC-DAD. The anticancer effects were assessed using AGS (gastric cancer), SKOV-3 (ovarian cancer), and HeLa S3 (cervical adenocarcinoma) cell lines, along with normal human fibroblasts, employing the MTT assay.

Polyphenol profiling revealed time-dependent variations, with the highest compound concentration observed on day 10. Eighteen compounds, primarily procyanidins and their derivatives, were identified. Catechin content peaked on day 20 (390.44 mg/100 g DM). Notably, *S. henryi* extracts exhibited significant anticancer activity, particularly against cervical and gastric cancer cells. These findings highlight the potential of bioreactor-cultivated *S. henryi* as a valuable source of bioactive polyphenols with promising anticancer properties, supporting its further investigation for pharmaceutical applications.

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P83 Hepatoprotective effect of a polar-lipophilic preparation from the root of *Eleutherococcus senticosus* (Rupr. & Maxim.) Maxim enriched with naringenin

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Adaptogens are natural substances that enhance an organism's ability to cope with stress and environmental changes. *Eleutherococcus senticosus* is widely studied for its adaptogenic properties. This study was aimed to evaluate the safety profile (hepatoprotection, blood parameters) of a novel polar-lipophilic extract from *E. senticosus* roots with added naringenin to support the use of this species in folk medicine and in a modern phytotherapy in the EU's countries [1, 2].

The combination of hydrophilic and lipophilic fractions may improve the bioavailability and therapeutic efficacy. *In vivo* experiments aim to assess the safety and potential application of the extract in liver-related deficiencies. The findings may provide scientific evidence supporting the effectiveness of this novel plant-based formulation. Preliminary findings indicated that the extract did exhibit a significant hepatoprotective effect. In the control group, which received silymarin at a dose of 50 mg/kg body weight, as well as in the study group that was treated with AdaptES at a dose of 500 mg/kg body weight, a lower level of liver enzymes and less liver damage were observed compared to the group that received paracetamol at a dose of 300 mg/kg body weight. The reduction in transaminase levels (ALT, AST) and other markers of liver damage, such as GGTP, GLDH, and alkaline phosphatase, indicates their potential hepatoprotective properties.

The study provides new insights into the therapeutic potential of *E. senticosus* roots extract. The combination of hydrophilic and lipophilic fractions with naringenin offers innovative prospects for *E. senticosus*-based drugs, highlighting its application in liver-related disorders.

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P84 *Aralia racemosa* L. – source of compounds with broad biological activity

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Aralia racemosa L., commonly known as American spikenard, is a herbaceous plant native to North America. In traditional Native American medicine, it was used to support cardiovascular function and overall vitality [1]. The plant is rich in bioactive compounds, including saponins, flavonoids, and phenolic acids, which may contribute to a broad spectrum of biological activities [2, 3]. The aim of this study was to characterize the chemical composition of *A. racemosa* root and leaves, and evaluate its anti-enzymatic and antioxidant potential.

Extracts from the root of *A. racemosa* exhibit strong antioxidant and anti-enzymatic properties, including activity against hyaluronidase, COX, LOX, and AChE. In particular, the methanol and acetone extracts showed the strongest activity in relation to ABTS and DPPH radicals, chelation of Fe²⁺ ions, and metal reduction in the FRAP and CUPRAC assays. An interesting observation was the stronger activity of the extracts against hyaluronidase compared to the positive control – escin. In contrast, extracts from the leaves showed significantly weaker antioxidant and anti-enzymatic activities.

The results suggest that *A. racemosa* may be an effective aid in the prevention of lifestyle diseases due to its antioxidant, anti-inflammatory, and metabolic properties. Further research is necessary to fully understand the mechanism of action.

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P85 Enhancing the antimicrobial efficacy of culinary herbs and spices by experimental design

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Herbs and spices are widely used across different populations, serving both culinary and medicinal purposes, particularly in sub-Saharan Africa [1]. Although herbs and spices are often used in combination, limited studies have explored their interactive antimicrobial effect [2, 3]. This study investigated the antimicrobial activity of 17 culinary herbs and spices (crude extracts and essential oils), individually, followed by 1:1 combinations of active extracts against six common foodborne pathogens. The minimum inhibitory concentrations (MIC) were determined using the broth microdilution assay. The design of experiments (DOE) approach was subsequently employed in MODDE 9.1® software, to optimize the experimental runs so as to identify the most effective antimicrobial interaction. Phytochemical profiling of the most active extracts was achieved using ultra-performance liquid chromatography coupled to mass spectrometry (UPLC-MS) analysis. The results demonstrated that combining *Rosmarinus officinalis* with either *Syzygium aromaticum*, or *Salvia officinalis* meth-

anol extracts produced synergistic antimicrobial effects towards *Bacillus cereus* with Σ FIC = 0.25 mg/ml and 0.31 mg/ml, respectively. The DOE predicted that a combination of higher ratios of *R. officinalis* (59.5%), higher ratios of *S. officinalis* (40%), and lower ratios of *S. aromaticum* (0.5%) would produce the best antimicrobial effect with MIC = 0.17 mg/ml. This prediction was experimentally confirmed, with a strong correlation ($r = 0.73$) [4] between predicted and experimental MIC values, leading to the identification of an optimal antimicrobial combination. The combination of *R. officinalis* (56%) and *S. officinalis* (44%) produced the antimicrobial effect (MIC = 0.19 mg/ml) which can be recommended for future studies.

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P86 Cape Verde endemic medicinal plants: chemical characterization and assessment of antitumoral potential of selected species in colorectal cancer cells

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Colorectal cancer (CRC) is the 3rd most common worldwide, associated with high mortality rates on both genders [1]. Inherited forms of CRC represent only 5–10% of cases, so the importance of environmental factors in CRC development is a growing research focus.

Currently, more than 60% of the available anticancer drugs in the clinic are derived from natural sources, so the Cape Verde flora, with 92 endemic species widely used in traditional medicine, although without scientific validation, are a potential source of antitumoral compounds [2].

For this study, we selected three species: *Artemisia gorgonum* Webb (AG), *Lavandula rotundifolia* Benth. (LR) and *Daucus carota* subsp. *annuus* (Bég.) Mart.Flores, D.M.Spooner & M.B.Crespo (DC). Infusion (I), decoction (D) and hydroalcoholic extracts (EtOH 80%) were prepared to mimic the preparations made by traditional healers.

The extracts were chemically characterized by HPLC-ESI-MSn and ATR-FTIR was used to characterize the presence of functional groups and to identify specific fingerprint regions. Cytotoxicity of these extracts was assessed *in vitro* (0.05–1.0 mg/mL) using the CRC cell line WiDr and the normal human colon tissue cell line CCD-841, by measuring cell viability (detecting the amount of ATP) and cell mass (sulforhodamine B assay).

EtOH 80% extracts of AG and LR showed selective cytotoxic profile for tumoral cells, so flow cytometry was performed to evaluate the impact on cell cycle arrest, and micro-FTIR to evaluate the impact of the compounds on cell's behaviour and environment. The evaluation of best concentrations' effect in a 3D *in vitro* spheroid cell model is ongoing.

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P87 Investigation of five selected *Salix* species against *Mycobacterium smegmatis*, a model bacterium for tuberculosis

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Tuberculosis (TB) caused by *Mycobacterium tuberculosis* is one of the leading causes of mortality globally with emerging drug-resistance [1, 2]. *Salix* species which are used for cough, lung problem and fever [3] could be good sources of new antimycobacterial compounds to combat TB. In this study, methanol, cold water and hot water extracts of the twigs of five *Salix* spp. were screened against *Mycobacterium smegmatis*. Also, some commercial compounds present in *Salix* species were tested against *M. smegmatis*. Agar diffusion and microplate methods were used to evaluate the antimycobacterial activity of the extracts and compounds.

Methanol and cold-water twig extracts of *S. caprea* were significantly active against *M. smegmatis* with growth inhibition zone diameters of 23.5 mm and 20.2 mm respectively. However, none of the twig extracts of *S. aurita*, *S. x pendulina*, *S. phylicifolia*, and *S. starkeana* showed activity against *M. smegmatis*. The commercial compounds tested were devoid of antimycobacterial activity at 1 mg/ml concentration. UPLC-PDA-QTOF/MS (ESI- mode) investigation of the *S. caprea* twig methanol extract revealed the presence of catechin (m/z 289.0709), procyanidin B1 (m/z 577.1344), salicin-7-sulfate (m/z 365.0538), naringenin (m/z 271.0599), luteolin (m/z 285.0391), salicortin (m/z 423.1288), taxifolin (m/z 303.0500) and salicin (m/z 285.0977) as the major compounds in the twig extracts of *S. caprea*. This study is the first report on the antimycobacterial activity of these selected *Salix* species. The results indicate that *S. caprea* could contain anti-TB agents to curb the increasingly drug resistant TB strains.

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P88 Are natural polyphenols or their colonic metabolites responsible for antiplatelet effects in humans?

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Epidemiological studies have linked a higher intake of polyphenols with a lower incidence of cardiovascular diseases including ischemic stroke [1]. As most polyphenols have low bioavailability [2], their colonic metabolites can be responsible for the effect.

The aim was to test if parent (iso)flavonoids or their known colonic metabolites can modify platelet aggregation at biologically achievable concentrations and to assess the mechanism of action.

A series of polyphenols, their metabolites and related derivatives were incubated with whole human blood and impedance aggregometry was used to investigate platelet aggregation. Platelet activity was further tested by additional methods (standard turbidimetry, ELISA). Healthy controls as well as patients suffering from hypercholesterolemia and diabetes, and geriatric polymorbid patients were included in the testing.

Tectorigenin, found in *Belamcanda chinensis*, was with an IC₅₀ of 7.5 μM the most active of the parent compounds. 4-methylcatechol (4MC), a metabolite of several polyphenols, was with an IC₅₀ of 3 μM even about one order of magnitude more potent than the standard antiplatelet drug acetylsalicylic acid (aspirin). This result was confirmed in generally healthy individuals [3] and newly in patients. Mechanistic experiments revealed 4MC to uncouple cyclooxygenase 1 and thromboxane synthase in synthesis of thromboxane A₂, a potent platelet signalling molecule.

Polyphenolic metabolites, in particular 4MC, seem to be involved in positive cardiovascular effects of flavonoid rich diet rather than parent compounds.

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P89 Biosynthesis and antidiabetic effect of zinc oxide nanoparticles using leaf extract of *Olea africana*

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Nanotechnology has recently appeared as an important study subject in modern material sciences. Green synthesis of nanoparticles has gained the attention of researchers because of its integral characteristics such as effectiveness, eco-friendliness, and low cost. Therefore, the present study used an environmentally friendly green synthesis method to produce zinc oxide nanoparticles

from *Olea africana* plant extract. Furthermore, the antioxidant and antidiabetic potential of the ZnO NPs was determined. The transmission electron microscopy displayed rod-like, spherical, and hexagonal shape of the biosynthesized ZnO NPs, with the particles ranging from 20–80 nm. The surface morphology was determined through scanning electron microscopy (SEM). The energy-dispersive X-ray spectroscopy (EDX) study showed that the synthesized ZnO NPs showed a high amount of zinc (55.67%), carbon (16.54%) and oxygen (22.29%) with a low amount of nitrogen (5.3%) and sulphur (0.15%). The Fourier transform infrared (FTIR) peak was found to be at 513.31 cm^{-1} . The biosynthesized ZnO NPs exhibited moderate scavenging capacity against DPPH (47.8% at 100 $\mu\text{g/mL}$) and nitric oxide (45.2% at 25 $\mu\text{g/mL}$). Conversely, the nanoparticles demonstrated high hydroxyl radical scavenging with a maximum scavenging rate of 92.3% (100 $\mu\text{g/mL}$). Additionally, the nanoparticles showed increased α -glucosidase and α -amylase inhibition activity at 53.9% (50 $\mu\text{g/mL}$) and 77.4% (12.5 $\mu\text{g/mL}$), respectively. The results of this study suggest that ZnO NPs biosynthesized from *Olea africana* leaf extract could potentially inhibit post-prandial hyperglycaemia and alleviate oxidative stress. However, further scientific validation is required for the therapeutic use of the biosynthesized ZnO NPs [4, 5].

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P90 Influence of *Agrimonia eupatoria* L. extracts on some aspects of wound healing

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Agrimonia eupatoria L. (Rosaceae) is an herb widely used in traditional medicine. The HMPC's conclusions on using agrimony medicines refer to the relief of mild diarrhea, oral, throat and skin inflammation, and wounds. In previous studies, we established that agrimony water extract possesses a rich polyphenolic composition, displaying remarkable antioxidant properties. Our research demonstrated its potential clinical utility in preventing and managing cardiovascular complications linked to diabetes and enhancing skin wound healing in rats [1, 2].

Aim of the study was determination of antimicrobial activity of *Agrimonia eupatoria* L. extracts by microdilution method and isothermal microcalorimetry, and evaluation of their influence on some aspects of wound healing.

Lyophilized water infusion of *Agrimonia eupatoria* L. was prepared in accordance with Pharmacopoeia Bohemoslovaca 4 [3]. 1g of agrimony lyophilisate was dissolved in water and divided between chloroform, butanol, leaving a water portion. It was divided in the ratio: chloroform < 1.5%, butanol about 37% and water content about 61%. All portions were analysed by HPLC/DAD/MS with significant differences in composition, antimicrobial activity and toxicity on HDF and HaCaT cells.

Our results revealed that the aqueous fraction of the water lyophilisate shows better stimulating activity in the medium concentration range and at the same time lower toxicity at high tested concentrations compared to the butanol fraction of the water lyophilisate, both in keratinocytes and fibroblasts. The stronger antimicrobial activity against gram positive bacteria was observed in the butanolic fraction of lyophilized water infusion. Gram negative bacteria exhibit stronger resistance to the tested extracts.

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P91 Cyclooxygenase and aromatase inhibitory activity of *Bergenia crassifolia* (L.) Fritsch leaf, rhizome and flower extracts

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Various plant parts of *Bergenia crassifolia* (L.) Fritsch (Saxifragaceae) are utilized by the traditional medicine systems of Mongolia, China and Russia for the treatment of a multitude of inflammatory diseases. Reported pharmacological activities include antimicrobial, anticancer and anti-inflammatory effects [1].

The aim of this ongoing study is to evaluate the anti-inflammatory and anti-aromatase activity of *Bergenia crassifolia*. In an initial screening, methanolic extracts of leaves, rhizomes and flowers were found to be strongly active in COX-1/-2, as well as in aromatase (CYP19A1) enzyme inhibition assays [2, 3]. For a detailed investigation of the active principles, we successively extracted the plant material with *n*-hexane, DCM, ethyl acetate, methanol and water by accelerated solvent extraction (ASE) and tested these extracts in both assays (50 $\mu\text{g/mL}$).

Besides the water extract, all other rhizome extracts showed very high levels of activity in the aromatase inhibition assay (close to 100% inhibition). With respect to flowers and leaves, DCM and ethyl acetate extracts had the highest inhibitory effect.

In the COX inhibition assay, all rhizome extracts, besides the water extract, showed high levels of both COX-1 and -2 inhibition. Similarly, lipophilic flower extracts inhibited both enzymes albeit with significantly higher levels of COX-2 than COX-1 inhibition. The lipophilic leaf extracts displayed only moderate levels of COX-1/-2 enzyme inhibition.

Phytochemical investigations comparing the different extracts and identifying the constituents responsible for the observed activities are in progress.

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The process was monitored by determining total saponins using HPLC-DAD and UHPLC-MS (Full MS), while QS-21 isomers were analyzed by UHPLC-MS (SIM, m/z 1987). Preliminary LC-MS data suggest the presence of four QS-21-like compounds in Qb leaves [1–3]. Additionally, total phenolic compounds were determined using Folin-Ciocalteu method.

Two high-value extracts were obtained: one enriched in saponins and another in phenolics. In both cases, P2 was more efficient than P1. Although this process produces saponins of intermediate purity, the resulting product could have direct applications or serve as a starting material for more efficient purification of pure compounds.

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P97 Effect of salicylic acid on bioactive compound variability in *in vitro* plantlets of *Hypericum perforatum* L.

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This study aimed to investigate *in vitro* shoot regeneration, the elicitation of salicylic acid (SA), antioxidant activity, and HPLC analyses performed on *Hypericum perforatum*. The highest shoot regeneration (86 %) was achieved using Linsmaier & Skoog (LS) medium containing 1 mg/L benzylaminopurine. Elicitation was applied to *in vitro* plantlets with different doses of SA (0.1, 0.05, and 0.01 mg/L). Total phenolic content, DPPH activity, and HPLC analyses were conducted on wild plants, those grown in the greenhouse, and the elicited plantlets. The highest total phenolic content and DPPH activity were obtained from wild leaf plants. The total phenolic content for *in vitro* plantlets increased by 1.2 times with 0.05 mg/L SA compared to the control (24.9 mg GAE/g extract). In elicitor applications, 0.01 mg/L SA exhibited a DPPH radical scavenging effect that was 1.7 times greater than the control (92.8 mg TE/g extract). Additionally, pseudohypericin, chlorogenic acid, hyperoside, quercetin, and quercitrin were analyzed in the plantlets elicited by SA during HPLC analysis. Hypericin and pseudohypericin were detected at the highest levels in leaves grown in greenhouses. In conclusion, the nutrient medium containing 0.05 mg/L SA produced more pseudohypericin than the control group. Moreover, the amount of chlorogenic acid decreased, and hyperoside was found at very low levels in the 0.1 mg/L SA treatment. Catechin, quercetin, and quercitrin compounds were produced in lower quantities than the control in all groups treated with 0.05 mg/L SA, while these compounds were produced in higher amounts in the other two groups (0.1 and 0.01 mg/L).

P98 Phytochemical investigation of *Cornus sanguinea*: Unreported phenolic acids and evaluation of antioxidant activity

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Cornus sanguinea L. (common dogwood) is a small tree with leaves showing a characteristic dark red colour in senescence [1]. The drupes are traditionally used as a remedy for diarrhea, stomachaches, sore throats, and gastrointestinal disorders, and roots are used as a febrifuge [2]. The fruits and the leaves showed antibacterial and antiseptic properties [1, 2]. To highlight the metabolites responsible of the biological activity, the methanol extract of *C. sanguinea* whole plant was investigated. An initial LC-MS profile of the extract was performed by UHPLC-ESI/QOrbitrap in negative ion mode. The careful analysis of accurate masses, molecular formula and ESI/MSⁿ spectra allowed us to identify 27 main compounds corresponding to phenolic acids, megastigmanes, flavonoids, a benzofuran and oxylipins. The MeOH extract was fractionated on Sephadex LH-20 and the obtained fractions were purified by HPLC-UV yielding compounds 1–25, of which structures were determined by 1D- and 2D- NMR experiments. Additionally, two oxylipins were tentatively identified by LC-MS (26–27). The chemical investigation yielded four previously unreported compounds: one megastigmane derivative and three phenolic acid derivatives. To determine *in vitro* antioxidant activity, ROS inhibition analysis was performed by DHR assay on HaCat keratinocytes cell line pre-treated with the extract. The extract displayed statistically significant activity in the range 32–125 µg/ml in the HaCat cells stimulated with 2mM H₂O₂. The phytochemical investigation of *C. sanguinea* has highlighted how the antioxidant activity reported in literature may be attributable to the presence of metabolites such as flavonoids and phenolics, among which the phenolic acid derivatives never reported before.

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P99 Anti-obesity potential of sparalide b isolated from *Sparassis crispa* via activating lipolysis in 3T3-L1 cells

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Obesity is a global health concern and a major risk factor for metabolic syndromes such as type 2 diabetes, cardiovascular diseases, and fatty liver [1]. With increasing awareness of the adverse effects of current pharmacological therapies, demand for safe and natural anti-obesity agents has grown. In this study, we evaluated the anti-obesity potential of *Sparassis crispa*, an edible and medicinal mushroom known for its immunomodulatory properties, with a particular focus on its bioactive compound, Sparalide B [2]. A 30 % ethanol

extract of *S. crispa* significantly inhibited lipid accumulation and adipocyte differentiation in 3T3-L1 preadipocyte cells. The extract showed superior activity compared to *Cissus* extract (CQR-300), a well-known functional ingredient for fat reduction. Sparalide B was successfully isolated through ethyl acetate fractionation, column chromatography, and high-performance liquid chromatography (HPLC). Its chemical structure was identified using ESI-MS, ¹H-NMR, and ¹³C-NMR spectroscopic techniques. Mechanistically, Sparalide B suppressed the expression of major adipogenic transcription factors, PPAR γ and C/EBP α , while activating the β -catenin signaling pathway. Additionally, it downregulated perilipin-1, a lipid-droplet coating protein, thereby promoting lipolysis and reducing intracellular lipid stability. Importantly, the compound exhibited no cytotoxicity at effective doses, indicating its safety for potential therapeutic use. These findings suggest that Sparalide B from *S. crispa* is a promising candidate for development as a functional food ingredient pharmaceutical agent targeting obesity.

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P100 Green synthesis of unique curcumin derivatives using *Trametes versicolor*

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Curcumin, the primary bioactive compound in *Curcuma longa* (turmeric), has attracted substantial scientific interest due to its diverse therapeutic potential. This polyphenol exhibits remarkable biological activities, most notably its potent antioxidant and anti-inflammatory properties [1]. Despite its numerous potential benefits, curcumin's effectiveness is limited by its poor bioavailability, rapid metabolism, and quick elimination from the body [1]. In the present work, the biotransformation of curcumin was investigated using fungal enzymes with the proposal to generate original bioactive derivatives [2]. Curcumin was incubated with the laccases of the saprophytic fungi *Trametes versicolor*. This chemoenzymatic synthesis was optimized through a systematic approach, beginning with analytical-scale reactions (1 mg/mL) conducted under varied conditions (temperatures: 25–35 °C, pH: 3.0–7.0, enzyme concentrations: 5–50 U/mL). Reaction progress was monitored in real-time using UHPLC-HRMS to confirm complete substrate conversion (>99%) and derivative formation. The optimized process was then scaled up to 1 g of curcumin, with HPLC-PDA-ELSD monitoring. Purification was achieved via high-resolution semi-preparative HPLC combined with dry load injection [3]. Notably, geometric transfer methods ensured chromatographic consistency across scales, maintaining matching selectivity profiles, minimal retention time prediction deviations, and identical elution orders between analytical and preparative systems [4]. This streamlined method enabled the single-step synthesis of a series of novel curcumin derivatives through phenoxy radical coupling reactions [5]. Structural elucidation was performed through comprehensive NMR and HRMS analysis. The isolated

compounds were submitted to a series of biological assays including Wnt inhibition and cytotoxic activity against breast cancer cells lines.

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P101 In depth phytochemical investigation of the roots of *Glycyrrhiza asymmetrica* guided by LCESI/HR/MS/MS

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The roots of some *Glycyrrhiza* L. species like *G. glabra* (liquorice) have long been used to treat respiratory, gastrointestinal, cardiovascular, and genital-urinary system diseases in different folk medicines [1]. The genus *Glycyrrhiza* contains many bioactive secondary metabolites including triterpene saponins, flavonoids, and coumarins, which are responsible for hepatoprotective, neuroprotective, anti-inflammatory, antimicrobial, antiviral, anti-protozoal, anticancer and antihyperglycemic effects [2]. In our very recent study, 23 secondary metabolites, including nine unreported compounds having dihydroaurone-3-enolic acid (licoaurones A and B), isoflavan (hydroxypreglabridin), isoflavanone (cyclodeoxykievitone), flavanone-3-ol (glycyasymmetrol), and isoprenylated bi-isoflavan (glycyasymmetrica A–D) skeletons were isolated from the roots of *G. asymmetrica* Hub.-Mor., an endemic species to Türkiye [3]. With the aim of obtaining an in-depth knowledge of the phytoconstituents of the ethyl acetate extract of the roots of *G. asymmetrica*, LC-ESI/(HR)QExactive/MS/MS guided isolation was carried out. Chromatographic techniques such as Sephadex LH-20 column chromatography and semi-preparative HPLC were utilized for the fractionation and purification. Structures of the isolates were unambiguously characterized by 1D- and 2D-NMR, as well as HRESIMS experiments. As a result, a total of 41 compounds were identified belonging to stilbene, dihydroaurone, chalcone, flavanone, flavanone-3-ol, isoflavan, isoflavone, isoflavanone, isoflavan, pterocarpin, and bi-isoflavan chemical classes.

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P102 Exploring silver skin coffee by-products as sustainable alternatives for *Rhipicephalus (Boophilus) microplus* ectoparasite control

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Livestock plays a crucial role in food production, however, the increasing demand for animal-derived products has contributed to the proliferation of the tick *Rhipicephalus (Boophilus) microplus*, leading to significant economic losses and disease transmission in livestock. The excessive use of chemical acaricides has resulted in parasite resistance, making control increasingly difficult. Phytotherapeutic acaricides have emerged as a promising solution due to their lower toxicity and reduced risk of resistance. Coffee (*Coffea arabica* L.) is one of the most consumed beverages globally and is known for its medicinal properties, including psychoactive, antioxidant and stimulant effects. However, large-scale coffee production generates waste, leading to research into new applications for its by-products, such as silver skin. Recent studies suggest that these wastes may possess acaricidal properties, opening up possibilities for sustainable pest control. To evaluate the *in vitro* larvicidal effect of the hydroethanolic extract (7:3) on *R. (B.) microplus* larvae, the larval package test (LPT) was performed using concentrations of 12.5, 25, 50 and 100 mg/mL, diluted in Milli-Q water and 2 % Tween. The negative control was Milli-Q water and 2 % Tween. The extract demonstrated activity at all concentrations tested, reaching a larval mortality rate of 2.86 % at the highest concentration. These studies reinforce the potential of by-products as an innovative and environmentally friendly alternative for ectoparasite control, allowing further investigations to advance towards product development. The use of natural compounds can help minimize the risks associated with chemical acaricides, such as toxicity to non-target organisms and contamination of the ecosystem.

P103 Antiproliferative and antioxidant activities of *Zephyranthes robusta* (Herb. ex Sweet) Baker, a Brazilian Amaryllidaceae

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Among medicinally significant botanical families, Amaryllidaceae ranks among the 20 most therapeutically relevant due to its unique alkaloids, which exhibit diverse biological activities, including antitumor and cytotoxic effects [1]. *Zephyranthes robusta* (Herb. ex Sweet) Baker is native to Brazil, although it is not endemic. This study aimed to investigate the biological activities of the secondary metabolites, particularly alkaloids, in the bulb and root of *Z. robusta*. Acid-base partitions were prepared from the crude methanolic extract from the plant's bulb and root, resulting in five samples. Antioxidant activity was evaluated using ABTS, DPPH, and iron chelating assays. Although overall antioxidant activity was not optimal, the best results were observed in the basic ethyl acetate fraction (AB) for the ABTS assay (IC₅₀ 122.8 µg/mL) and in the ethyl acetate with methanol in basic medium fraction (AM) for the iron chelation test (IC₅₀ 121.3 µg/mL). Cytotoxicity assays using the MTT method on MCF-7 and MCF-10A cell lines revealed dose-dependent and time-dependent behavior. In the MCF-7 cell line, low IC₅₀ values were recorded for the AB (10.95 µg/mL, 72 h) and AM (9.93 µg/mL, 72 h) fractions, whereas cell viability in the MCF-10A

cell line remained high [2]. Furthermore, selectivity index calculations indicated that the crude extract (EB), ethyl acetate with methanol in acid medium AA, AB, and AM samples exhibited selectivity, with the AB fraction at 72 h showing the highest index (SI 102.283).

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P104 Metabolomics of *Andrographis paniculata* Burm. F. extracts by a combined LC-MS and NMR approach and evaluation of acetylcholinesterase and tyrosinase inhibitory activity

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Andrographis paniculata Burm. F. (Acanthaceae) is also called Kalmegh or “King of Bitters”, because of its intensely bitter tasting leaves [1]. Since ancient times, it has been widely recognized for its therapeutic properties and has attracted the scientific and medical communities’ attention [2]. The aerial parts represent the part mostly used for their antibacterial, antifungal, antiviral, choleric, hypoglycemic, hypocholesterolemic, and adaptogenic effects [3]. The phytochemical profile of *A. paniculata* includes, as the main chemical constituents, diterpenoids and flavonoids, responsible for the biological activities [1]. Among the diterpenoids, andrographolide is the major one in terms of bioactive properties and abundance. Several studies have shown that andrographolide possesses pharmacological effects on the CNS [4]. Based on the health effects of *A. paniculata* aerial parts and its phytoconstituents, this work aims at investigating how the extraction methods (maceration, UAE and SLDE), using different mixtures (100 % EtOH, 75 % EtOH/H₂O, and 50 % EtOH/H₂O) affect the chemical profile and the biological activity of the extracts. The definition of their chemical profile was performed by a combined approach based on LC-HR-MS and NMR analysis, allowing us to identify both primary and secondary metabolites. The comparison of the chemical profiles was performed using a multivariate statistical approach such as Principal Component Analysis (PCA). The tyrosinase and AChE inhibition activities of extracts and all compounds were tested by spectrophotometric assays, providing further information about the potential CNS effects. These results were evaluated with respect to the metabolomics data by Partial Least-Squares Discriminant Analysis (PLS-DA).

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P105 Study on volatile compounds of *Artemisia caerulescens* from Croatia

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Halophytes are plant species that are adapted to areas with high salinity and can survive in salty habitats such as the sea, seashore, salt marshes, lakes, etc. In Croatia, halophytes grow not only by the sea, but also on the coast in the spray zone and in some places further away from the sea, where the salinity is particularly high due to strong winds and the soil is therefore salty. Plants that live in these areas have special adaptation mechanisms. These include a complex network of biochemical mechanisms and a range of bioactive molecules. In the Mediterranean region, rural communities use halophytes both as food and as a source of health benefits. The aim of this study was to determine the volatile compounds of *Artemisia caerulescens* from the Pantan Reserve, Croatia. The essential oil was isolated from air-dried above-ground plant material by hydrodistillation. The aqueous layer that remains after isolation of the essential oil is the hydrolate.

The chemical composition of the volatile compounds was determined by the coupled technique of gas chromatography-mass spectrometry (GC-MS) using the HP-5MS column. Headspace solid phase microextraction (HS-SPME) was used to isolate the volatile components from the hydrolate. The main components of the essential oil and hydrolate were camphor (41.6 and 52.1 %, respectively) and α -thujone (34.2 and 26.8 %, respectively). Several manuscripts on the chemical composition of the essential oil of this plant have been published so far, but this is the first manuscript on the chemical composition of the hydrolate.

P106 Influence of antibiotics on the ellagic acid biotransformation in ex vivo incubations with gut microbiota

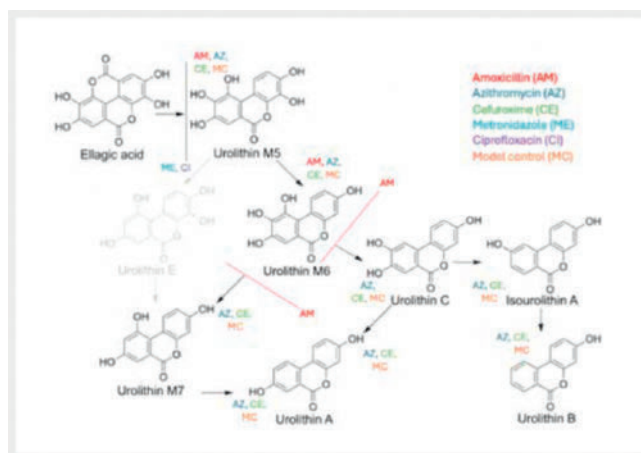
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The activity of orally taken ellagitannins is mostly dependent on the ability of individual gut microbiota to produce urolithins [1]. Thus, regardless of the urolithin metabolite, the antibiotics might influence species responsible for the ellagic acid biotransformation [2]. The study aimed to investigate how various antibiotics influence the urolithins' production in anaerobic co-incubations with gut microbiota and ellagic acid, used as the substrate. The experiments were conducted using fecal samples from different donors. The samples were taken at several time points and analyzed using LC-HRMS.

The biotransformation was most often halted at the step of the conversion of ellagic acid to urolithin M5, and urolithin M6 to urolithins M7 and C (► Fig. 1). The antibiotics that caused the inhibition of urolithin production in most of the experiments were amoxicillin and ciprofloxacin. The results showed high inter-individual variability, and no particular pattern was observed for specific metabolites.



► Fig. 1 Ellagic acid biotransformation pathway – model control and incubations with antibiotics. Example of results for a single urolithin metabolite B donor. Urolithin E was not observed.

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P107 Production of triterpenoid saponin glycosides in *Bacopa monnieri* (L.) Wettst. through tissue culture with elicitor and precursor treatments

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Triterpenoid saponin glycosides are bioactive compounds found in *Bacopa monnieri* that enhance learning and memory functions [1, 2]. This study aimed to explore the effects of elicitors and precursors [3] on the production of these bioactive compounds in *B. monnieri* tissue culture. Methyl jasmonate and yeast extract significantly increased bioactive compound levels in all tested concentrations, with day 6 being the optimal duration for elicitor treatments. At concentrations of 200 μ M methyl jasmonate, the highest levels of total triterpenoid saponin glycosides was recorded at 26.24 ± 0.06 mg/g DW. Similarly, the combination of 100 μ M methyl jasmonate and 1 mg/mL yeast extract further enhanced total triterpenoid saponin glycosides to 27.89 ± 0.77 mg/g DW. When elicitor and precursor treatments were combined, the maximum total bioactive compound content reached 52.62 ± 0.19 mg/g DW using a combination of 1 mM L-alanine, 100 μ M L-phenylalanine, 100 μ M methyl jasmonate, and 1 mg/mL yeast extract. These findings demonstrate that the production of triterpenoid saponin glycosides can be effectively enhanced through elicitor and precursor treatments. These techniques offer a promising alternative approach for increasing bioactive compound yields and may provide value-added and effective raw materials for the herbal and food supplement industries.

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P108 Isolation and identification of antibacterial lead compounds against Methicillin-Resistant *Staphylococcus aureus* (MRSA) from propolis

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Discovery of new antibiotics is crucial to address the increasing threat of antimicrobial resistance (AMR) faced by the global health sector. Propolis is a bee product known for its diverse bioactive properties, including antimicrobial, anti-inflammatory, antioxidant, and wound-healing effects [1]. Propolis is considered an ancient healer and used as a natural remedy to cure infections and support overall health. Its antibacterial properties and potentials to address AMR make it an active research and exploration area [2, 3]. This study explores the antibacterial potential of Turkish and Nigerian propolis samples, focusing on their activity against Methicillin-resistant *Staphylococcus aureus* (MRSA), a notorious multidrug-resistant pathogen. Dichloromethane and methanolic extracts of both Turkish and Nigerian propolis samples revealed minimum inhibitory concentrations (MICs) in the range of 32–256 and 256–512 µg/mL, respectively against a panel of clinical isolates of MRSA strains. A bioassay-guided approach was used to isolate anti-MRSA compounds using a range of chromatographic techniques including vacuum liquid chromatography (VLC), solid phase extraction (SPE), and preparative thin layer chromatography (PTLC). Compounds have been characterized by ¹H NMR, ¹³C NMR, and 2D NMR spectroscopy. The compounds isolated from the dichloromethane extracts of Turkish propolis include chrysin, tectochrysin, pinocembrin, and caffeic acid derivatives. Further investigation into the mechanism of action of these bioactive compounds could facilitate the discovery of novel antibiotics and support global efforts to tackle antimicrobial resistance.

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P109 Underexplored potential of *Viburnum opulus* L. inflorescences: Phenolic profiling across flowering phenophases

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Viburnum opulus L., commonly known as guelder rose, is known for its medicinal properties, primarily attributed to its fruits and bark [1, 2]. However, the phenolic composition of its inflorescences remains inadequately explored. This study aims to elucidate the phenolic profiles of cultivated *V. opulus* inflorescences at different flowering stages, highlighting their potential as valuable sources of bioactive compounds. Inflorescence samples were collected during three distinct flowering stages: the beginning of flowering, massive flowering, and the end of flowering. Ethanol extracts were analyzed using high-performance liquid chromatography (HPLC) and spectrophotometric assays to evaluate the antioxidant activity. The results revealed significant variations in phenolic and antioxidant activity across the flowering stages. Notably, chlorogenic acid was the predominant compound during all flowering stages, constituting approximately 35–65 % of the total identified phenolics. Kaempferol, quercetin, and isorhamnetin derivatives predominated the flavonoid profile with significant differences during the flowering stages. The antioxidant activity during the beginning of flowering was determined by the amount of identified phenolic acids and, in certain species, tended to increase at the end of flowering due to the elevated flavonoid quantities. These findings suggest that the phenolic composition of *V. opulus* inflorescences is dynamic and stage-dependent. The high phenolic compound diversity in inflorescences suggests that they are an underutilized resource for pharmaceutical, nutraceutical, and functional food applications.

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P110 Isolation and characterization of novel withanolide-type compounds with anti-inflammatory activity from *Witheringia solanacea*

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The chemical composition of *Witheringia solanacea* has been scarcely studied [1], unlike other plants in the Solanaceae family, where numerous anti-inflammatory compounds, such as withanolides, have been identified [2]. Therefore, this study aimed to isolate novel compounds with a withanolide-type structure and anti-inflammatory activity. Two previously unreported compounds were isolated from a methanolic using liquid-liquid extraction and chromatographic separations on normal and reverse-phase columns. The molecular formula and structural elucidation were determined using high resolution mass spectrometry and one- and two-dimensional nuclear magnetic resonance spectroscopy. Both compounds were evaluated for their ability to inhibit inducible nitric oxide synthase (iNOS). Compounds 1 and 2 were successfully isolated and characterized, identified both as 18-norwithanolides-26-O-glucosides, with minimum inhibitory concentrations (iNOS) in RAW 264.7 cells of 11.6 ± 1 µM and 26.5 ± 1 µM, respectively.

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P111 Effect of ethanol concentration on hydroxychavicol and eugenol of fresh and dried *Piper betle* L. laves

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Piper betle L., a commonly used medicinal plant, contains bioactive compounds like hydroxychavicol (HC) and eugenol (EU), which provide various therapeutic benefits [1, 2]. However, previous studies on the ethanol (EtOH) extraction technique have been limited in exploring how different EtOH concentrations affect these compounds during both pre-treatment and post-treatment stages. This research aimed to examine the effects of fresh and dried *P. betle* L. leaves on the extraction of HC and EU using EtOH at varying concentrations. HPLC results showed that HC and EU were eluted sequentially with retention times of 16.4 and 20.2 min, respectively. The calibration curve had a linearity range of 12.5–400 µg/mL ($r^2 = 0.999$). All EtOH concentrations were able to extract HC and EU, except 40 % EtOH of fresh leaves. The 95 % EtOH gave the highest amounts of HC and EU, followed by 70 and 40 % EtOH extraction. Compared dry leaves to fresh leaves, higher amounts of HC and EU were found in the dried leaves. In addition, dry leaves extracted with 95 % EtOH produced the maximum amount 35.50 % of HC, while fresh leaves provided only 13.96 % of HC. The amounts of eugenol was similar from fresh leaves (1.88 %) and dry leaves (1.97 %) using 95 % EtOH. The finding indicated that dry leaves extracted with 70–95 % EtOH produced significantly higher amounts of HC than fresh leaves. In conclusion, using 70–95 % EtOH is the most effective approach for extracting HC and EU from *P. betle* L. leaves. This efficient, heat-free technique is suitable for various industrial applications.

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P112 Evaluation of the potential for immunogenic cell death induction of low molecular weight sulfated galactan in human breast cancer

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Sulfated galactan (SG) from *Gracilaria fisheri* has the potential for cancer cell therapy since its discovery [1, 2]. Our previous study showed that low molecular weight of SG (LMSG) could enhance biological activities, such as antioxidant activity [3]. In this study, we aimed to evaluate the anticancer activity of LMSG, particularly its ability to induce immunogenic cell death. LMSG, prepared using HCl-controlled degradation, was characterized by FTIR, NMR, and GPC analyses, while its anti-breast cancer activity was assessed using normal breast MCF-10A cells, as well as breast cancer MCF-7 and MDA-MB 231 cell cultures. Additionally, the expression of immunogenic cell death proteins (Fas-R, CRT, and MHC class-I) was determined after treatment with LMSG in MDA-MB 231 cells using qPCR, Western blot, and confocal microscopy assays. The results revealed that LMSG was not cytotoxic to normal breast MCF-10A cells, while it was slightly cytotoxic to human breast cancer MCF-7 and MDA-MB 231 cells. Cytotoxicity

in cancer cells, indicated by morphological changes, correlated with cell number reduction and cell death. The expression levels of Fas-R and CRT were up-regulated in the LMSG treatment group, similar to the positive doxorubicin control. However, the expression levels of MHC class-I did not change in any of the groups. In conclusion, our results suggest that LMSG has the potential to enhance the expression of immunogenic cell death in human breast cancer and could be used as an adjuvant for cancer cell therapy. Finally, the authors declare that they have no competing interests.

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P113 Anti-inflammatory activity of lupinifolin and *Derris reticulata* crude extract in lipopolysaccharide and recombinant receptor-binding domain of SAR-CoV-2 induced macrophage cells

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Derris reticulata has been used in traditional Thai medicines and uses for fever and skin diseases treatment [1]. This herb was reported as α -glucosidase inhibitory, antibacterial, and antioxidant [2]. The major compound of stem parts is lupinifolin, prenylated naringenin derivative. Currently, SAR-CoV-2 pandemic has been occurred which involved immunity and respiratory systems. From these pharmacological activities of this herb, we aimed to investigate anti-inflammatory activity using lipopolysaccharide (LPS) or receptor-binding domain (RBD) of SAR-CoV2 [3] induced nitric oxide (NO) production in RAW 264.7 macrophage cell line [4] and co-treatment with lupinifolin and stem crude extracts of *D. reticulata*. We used RT-PCR to investigate gene expression and proved mechanism of action. The results showed inhibition of LPS- and RBD-induced NO production were 50 µM lupinifolin (46.85 and 79.52 %, respectively) and 1000 µg/mL stem extract (66.42 and 77.61 %, respectively). Both LPS- and RBD-induced related inflammatory genes expression as inducible nitric oxide synthase (*iNOS*), cyclooxygenase-2 (*COX-2*), interleukin-6 (*IL-6*) and 5-lipoxygenase (*5-LOX*) genes in RAW 264.7 cells. All treatment samples significantly suppressed inflammatory genes expression from inducer. Stem extract down-regulated *iNOS*, *IL-6* and *5-LOX* genes from LPS-induction (2.2, 2.8 and 13.0-fold, respectively). RBD-induced *iNOS*, *COX-2*, *IL-6* and *5-LOX* genes expression were suppressed (1.9, 3.5, 7.5 and 3.6-fold, respectively). Lupinifolin inhibited *COX-2* gene from LPS induction (2.3-fold). Lupinifolin and stem crude extracts of *D. reticulata* might be used for anti-inflammation in macrophage. However, it is necessary to quantify and identify standard compounds in extracts, as well as conduct *in vivo* studies to investigate their effects.

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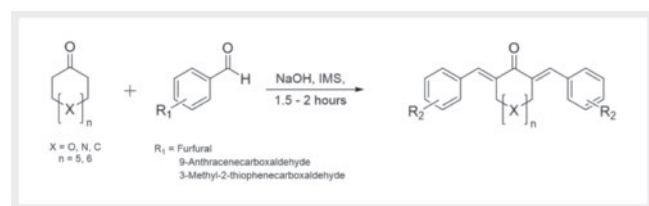
P114 Synthesis and evaluation for antimicrobial activity of curcumin derivatives and related compounds

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Antimicrobial resistance (AMR) is recognized by the World Health Organization as a top global health threat, with 4.95 million deaths attributed to AMR in 2019 [1]. The rise of resistant bacterial strains has rendered many conventional antibiotics ineffective, necessitating the search for alternative therapeutics. Curcumin, a bioactive diarylheptanoid from *Curcuma longa*, has been used medicinally for over 4,000 years [2]. It exhibits antimicrobial, anti-cancerous, and anti-Alzheimer's potential but has limited clinical application due to poor physicochemical and pharmacokinetic properties [3]. To address this, curcumin derivatives (CDs) were synthesized using the Claisen-Schmidt reaction, while the Suzuki coupling was explored to extend carbon side chains. A library of CDs were synthesized, guided by literature, prior research, and *in silico* predictions using SWISSADME. The compounds were characterized via NMR, IR, and MS. Antimicrobial activity was evaluated using disk diffusion and minimum inhibitory concentration (MIC) assays against Methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, and *Escherichia coli*. Positive inhibitory activity against *Pseudomonas aeruginosa* and MRSA was established by specific derivatives. Structure-activity relationship analysis highlighted enhanced activity in derivatives with substituted central rings, halogen substituents, or bulky side groups. Future research will focus on improving solubility and optimizing Suzuki reaction conditions for potential drug development (► Fig. 1).



► **Fig. 1** General reaction scheme for the synthesis of curcumin derivatives.

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P115 Triterpenoids and steroids in *Amelanchier × spicata* (Lam.) K. Koch leaves and fruits: Phytochemical composition and potential benefits

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Amelanchier × spicata, or dwarf serviceberry, is a deciduous shrub native to North America but invasive in parts of Northern and Central Europe, including Lithuania, Sweden and Finland. It spreads aggressively, outcompeting native vegetation. The leaves and fruits of the plant contain bioactive compounds, including triterpenoids and steroids, with potential health benefits and pharmacological properties [1, 2]. The aim of this work was to analyze the triterpenoid and steroid content in the cuticular waxes of the fruits and leaves of *A. × spicata*.

Leaves and fruits of *A. × spicata* were collected in western Lithuania in July 2023. The qualitative and quantitative analyses of steroid and triterpenoid fractions were performed using an Agilent Technologies 7890A gas chromatograph (GC-MS) with a 5975C mass selective detector, equipped with a 30 m × 0.25 mm i.d. HP-5MS UI column (0.25-μm film thickness), from Agilent Technologies and Perlan Technologies.

The cuticular waxes of *A. × spicata* leaves contained the same analytes as those of the fruits; however, their concentrations differed. The cuticular waxes were dominated by ursolic acid, with levels of 2024.76 μg/g DW in leaves and 299.05 μg/g DW in fruits, respectively. Sitosterol was the main compound in leaves (697.57 μg/g DW) and fruits (248.19 μg/g DW), nearly three times higher in the leaves. The dominant compound in the neutral triterpenoid class was β-amyrin, with levels of 1039.56 μg/g DW in leaves and 38.96 μg/g DW in fruits, respectively.

The higher concentration of triterpenoids in the leaves compared to the fruits, along with the distinct composition of compounds like isofucosterol in fruits and taraxerol in leaves, underscores the therapeutic potential of both plant parts.

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P116 Generation of original bioactive chalcones derivatives by fungal-assisted biotransformation

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Natural products (NPs) and their derivatives have long been a rich source of therapeutic drugs. Among the strategies for discovering new bioactive compounds, biotransformation stands out as a promising approach to generate structurally unique NP derivatives. This study aims to enhance the chemical diversity of conventional natural products through an innovative biotransformation approach. The approach leverages fungal enzyme mixtures—referred to as “fungal secretomes” in combination with purified enzymes such as laccases from *Trametes versicolor* [1]. Chalcones, a class of natural polyphenols with an open-chain flavonoid structure, were selected as ideal substrates for this transformation due to their wide distribution in plants and their central role in flavonoid and isoflavonoid biosynthesis [2]. Two representative chalcones – xanthohumol, found mainly in hops (*Humulus lupulus*), and butein, isolated from the lacquer tree (*Toxicodendron vernicifluum*) were selected based on their documented broad spectrum of biological activities, including anti-inflammatory, antimicrobial, and anticancer properties [3, 4]. Their hydroxyl and prenyl functional groups provide promising features for enzymatic modification and derivatization. Biotransformations were conducted on an analytical scale (1 mg/mL), with reaction parameters including temperature (25–35 °C), pH (4.0–7.0), and enzyme concentrations (0.075–1.5 U/mL) systematically optimized. Metabolite profiling was performed using UHPLC–PDA–ELSD–MS. Reactions yielding promising products were scaled up to the gram scale, and compounds were isolated via high-resolution preparative chromatography with dry load injection [5]. Structural elucidation was achieved through high-resolution mass spectrometry (HRMS), and nuclear magnetic resonance (NMR) spectroscopy. This strategy yielded a chemically diverse library of novel chalcone derivatives, which were evaluated for activity against multidrug-resistant bacterial strains, respiratory syncytial virus (RSV), and various cancer cell lines. Notably, several butein-derived compounds exhibited particularly promising preliminary antiviral activities.

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P117 Phytocompounds as potential inhibitors of p300-HIF1 α interaction: A structure-based screening and molecular dynamics simulation analysis

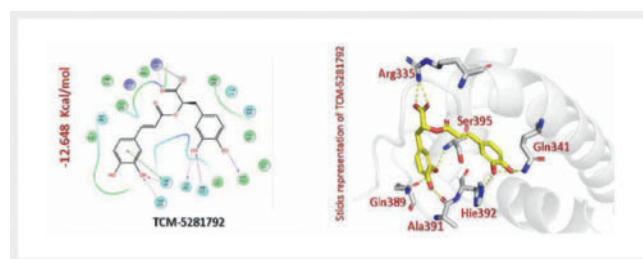
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Hypoxia plays a key role in cancer progression, mainly by stabilizing and activating hypoxia-inducible factor-1 (HIF-1) [1]. For HIF-1 to function under low oxygen conditions, it must interact with the transcriptional coactivator p300, a critical step for promoting cancer cell survival and adaptation in hypoxic environments [2, 3]. Consequently, this study aimed to use drug designing and molecular simulation techniques to screen phytochemical databases, including

Traditional Chinese and African Medicine sources, for compounds that could disrupt the p300/HIF-1 interaction. In this study, we identified potential compounds with high docking scores such as EA-176920 (-8.719), EA-46881231 (-8.642), SA-31161 (-9.580), SA-5280863 (-8.179), NE-5280362 (-10.287), NE-72276 (-9.017), NA-11210533 (-10.366), NA-11336960 (-7.818), TCM-5281792 (-12.648) and TCM-6441280 (-9.470 kcal/mol) as lead compounds. Furthermore, the compound with the highest docking score from each database (EA-176920, SA-31161, NE-5280362, NA-11210533, and TCM-5281792) was subjected to further analysis. The stable binding affinity of these compounds with p300 was confirmed by molecular simulation, binding free energy (-22.0020 kcal/mol, -25.4499 kcal/mol, -32.4530 kcal/mol, -33.9918 kcal/mol, and -57.7755 kcal/mol, respectively) and KD analysis. Moreover, the selected compounds followed the Lipinski rules with favorable ADMET properties like efficient intestinal absorption, high water solubility, and no toxicity. Our findings highlight the potential of natural compounds to target key protein-protein interactions in cancer and lay the groundwork for future *in vitro* and *in vivo* studies to explore their therapeutic potential. Specifically, disrupting the p300/HIF-1 interaction could interfere with hypoxia-driven pathways that promote tumor growth, angiogenesis, and metastasis offering a promising strategy to suppress cancer progression at the molecular level ► **Fig. 1**.



► **Fig. 1** The interaction analysis of lead phytocompounds and p300. The Fig. illustrates the 2D and 3D interaction mechanism of rosmarinic acid (TCM-5281792) and p300.

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P118 *In vitro* and *in silico* analysis of anticancer activity of terpenoids of *Dittrichia viscosa* (L.) Greuter essential oil

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There is a continuous global search for new anticancer agents from natural sources. In this study, the essential oils of *Dittrichia viscosa* (L.) Greuter leaves (DVEO) were extracted by hydrodistillation and chemically profiled using GC-

MS, identifying 25 compounds, mainly terpenoids. DVEO in vitro cytotoxicity assays against six human cancer cell lines (MDA-MB-231, A549, MCF7, SKOV3, HepG2, and HCT116) revealed strong antiproliferative effects (IC_{50} between 16.2 and 30 $\mu\text{g/mL}$), with selective safety against normal neonatal human fibroblasts (HDFn, IC_{50} 42 $\mu\text{g/mL}$) [1]. Virtual screening and network pharmacology analysis including ADMET screening, protein-protein interaction analysis, and molecular docking of the predominant compounds -11-hydroxy-eremophyl-6(7),9(10)-dien-8-one (30.56% area), 3,7,11-trimethyl-1,6,10-dodecatrien-3-ol (10.87%), and geraniol (17.93%) – identified MMP9, PTEN, and STAT3 as key targets [2, 3]. The lead compound, 11-hydroxy-eremophyl-6(7),9(10)-dien-8-one, showed a high binding affinity with MMP9 (–7.3 kcal/mol), PTEN (–7.0 kcal/mol), and STAT3 (–6.8 kcal/mol). The strong binding affinity of these compounds with the targets was confirmed through molecular dynamics simulation and binding free energy calculations (–36.61 kcal/mol), demonstrating stable binding, structural compactness, and residual fluctuations upon compound binding [3, 4]. Density Functional Theory (DFT) further confirmed the compound's electronic stability and reactivity [5]. These findings establish *D. viscosa* essential oil, particularly its prevalent terpenoids, as promising candidates for anticancer drug development.

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P119 Thirty-five steroidal glycosides from *Ornithogalum thyrsoides* bulbs and their cytotoxicity against human leukemia cells and human small-cell lung cancer cells

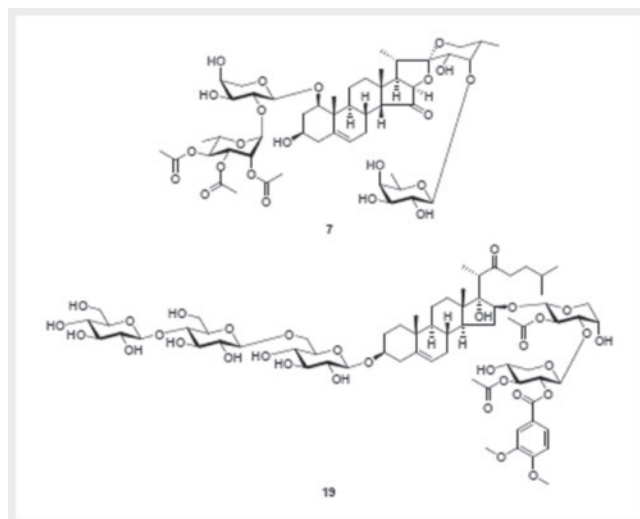
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The authors have previously reported several cholestane- and spirostan-type steroidal glycosides from *Ornithogalum thyrsoides* [1–4]. Since conventional TLC analysis of the extract of *O. thyrsoides* bulbs suggested the presence of unprecedented compounds, a comprehensive phytochemical investigation of *O. thyrsoides* bulbs was conducted, which resulted in the isolation of 35 steroidal glycosides (**1–35**), including 21 previously undescribed ones (**1–21**). The structures of **1–21** were determined mainly by NMR spectroscopic analyses. The isolated compounds were classified into three distinct groups: furostan-type, spirostan-type, and cholestane-type. Although the C/D-ring junction of the steroidal skeleton is generally *trans*-oriented, except for certain cardiotonic and pregnane-type steroidal derivatives, **7** was revealed to possess a *cis* C/D-ring junction (► Fig. 1). This is the first reported instance of such a configuration in spirostan-type steroidal derivatives, marking it as a finding of significant interest. Compounds **1–35** were evaluated for cytotoxicity against HL-60 human promyelocytic leukemia cells and SBC-3 human small-cell lung cancer cells. The isolated compounds exhibited cytotoxicity in a dose-dependent manner with IC_{50} values ranging from 0.00086 to 18 μM toward HL-60 cells, and from 0.00014 to 37 μM toward SBC-3 cells, respectively. Compound **19** (► Fig. 1), which demonstrated relatively potent cytotoxicity among the

novel compounds, induced apoptosis in HL-60 cells, concomitant with cell cycle arrest at the G2/M phase. In contrast, **19** caused oxidative stress-associated necrosis in SBC-3 cells. These findings indicate that **19** induces cell death through distinct mechanisms of action in HL-60 and SBC-3 cells, respectively.



► Fig. 1 Structures of **7** and **19**.

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P120 Anti-phytopathogenic potential of resinous exudates from plants of the genus *Adesmia*, Balsamiceae series

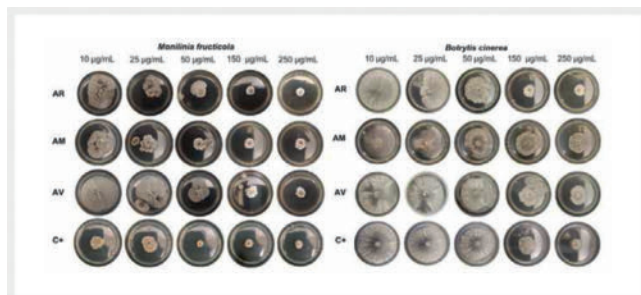
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Filamentous fungi, such as *Monilinia fructicola* and *Botrytis cinerea*, are challenging phytopathogens responsible for brown and gray rot in postharvest fruits, respectively. These fungi decrease marketability and cause economic losses estimated in millions of dollars [1]. There is a growing interest in finding non-toxic, nature-based alternatives, and resinous exudates from plants have a promising and unexplored potential. Plants of the genus *Adesmia*, Balsamiceae series, are known for their ethnopharmacological value and their abundant resin, which protects the plant against biotic and abiotic stress, acting as an antidesiccant, and defending it against microorganisms [2]. These natural products are valued for their high yield, easy extraction, and minimal solvent use, making them attractive for the agricultural industry. This study aimed to determine the composition of resinous exudates from three *Adesmia* species (AR, AM and AV) and their antifungal properties against *M. fructicola* and *B. cinerea*. Results showed that, although they were different in composition, the exudates

are rich in chalcones and other products from the shikimic acid pathway. Antifungal activity is illustrated in ► **Fig. 1**; AM showed a mean effective concentration (EC₅₀) of 19.71 ± 0.25 µg/mL in *M. fructicola*, followed by AR (33.72 ± 2.48 µg/mL). For *B. cinerea*, AR achieved 100% conidial inhibition with an EC₅₀ value of 60.64 ± 0.57 µg/mL, making it the most effective treatment. These findings suggest that resinous exudates have potential applications in controlling postharvest phytopathogens on agroindustry.



► **Fig. 1** Effects of resinous exudates (AR, AM and AV) and positive control BC-1000® (C+) on mycelial growth of *M. fructicola* and *B. cinerea*.

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P121 *Erythroxylum tortuosum* Mart. and its antidiabetic potential: Bridging sustainability and health

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Between 2003 and 2022, 12% of the Cerrado's native vegetation (24 million hectares) was lost, an area equivalent to the state of São Paulo. In 2023, the Cerrado biome surpassed the Amazon in deforestation [1]. Due to environmental degradation, it is classified as a *hotspot*, harbouring threatened biodiversity [2]. Thus, new initiatives for its conservation are essential. Brazilian flora represents a natural source of new products for sustainable development, especially medicinal plants, such as *Erythroxylum tortuosum* Mart. (Et), Erythroxylaceae, native to the Cerrado. This research was conducted in the Federal District's Conservation Units, investigating species used as antidiabetics. The ethanolic extract (EE) from the leaves was evaluated for inhibition of α-amylase and α-glucosidase (7.81–500 µg/mL) using colorimetric methods. EE fractions were subjected to column chromatography. Chemical characterization included TLC, HPLC-DAD, and NMR. In the bioassay, EE showed an IC₅₀ of 77.51 µg/mL for α-amylase, whereas the EtF3 fraction had an IC₅₀ of 39.50 µg/mL (acarbose, IC₅₀ = 4.23 µg/mL). For α-glucosidase, EE resulted in an IC₅₀ of 21.60 µg/mL, and EtF3, 9.11 µg/mL (deoxynojirimycin, IC₅₀ = 28.83 µg/mL). This suggests the inhibitory specificity of EE and its potential in controlling postprandial hyperglycemia [3]. The phytochemical and spectrometric procedures led to the identification of rutin and ombuin 3-O-rutinoside. These preliminary results are promising for bioguided studies aimed at identifying bioactive antidiabetic

ic compounds. Moreover, it reinforces the importance of biodiversity conservation.

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P122 Antimicrobial activity of *Lobelia brasiliensis* A.O.S.Vieira & G.J.Sheph.: A preliminary study

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Lobelia brasiliensis A.O.S.Vieira & G.J.Sheph. is endemic to the Federal District, and classified as rare in its natural habitat. This species predominantly occurs in wetland areas (*veredas*), being considered a bioindicator of water presence in the region. Although several *Lobelia* species have been extensively investigated regarding their phytochemical composition and potential pharmacological properties, *L. brasiliensis* remains poorly studied, with limited data available in scientific literature. Among the species of the genus, antimicrobial activities have been reported, which motivated the present study to evaluate the antimicrobial activity of *L. brasiliensis*. The assay was conducted after partitioning the crude hexane extract (He0) into five fractions according to their polarity. The antimicrobial activity was evaluated using the broth microdilution method [1] against *Escherichia coli*, *Staphylococcus aureus*, and *Mycobacterium smegmatis*. The extract and fractions were diluted in DMSO. The assays were conducted in volumes ranging from 0.1 to 0.2 mL, with concentrations varying from 0.03125 to 1 mg/mL (maximum concentration), by using the microdilution technique. None of the samples inhibited the bacterial growth of *S. aureus* and *E. coli*, even in the highest tested concentration. However, the hexane extract was able to inhibit *M. smegmatis* growth at concentrations higher than 0.5 mg/mL. These findings suggest promising prospects for antimicrobial agents derived from this plant species. Additional studies are required to confirm this activity, as well as against other bacterial species.

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P123 New insights into the carbohydrate composition of the red alga *Vertebrata lanosa*

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Vertebrata lanosa (L.) T.A. Christensen is a red alga commonly found at the North Atlantic coasts. Its extracts are applied as cosmetic ingredients [1], whereas the dried plant is consumed as spice in some regions of Europe. Previous phytochemical investigations revealed various secondary metabolites such as bromophenols, mycosporine-like amino acids or carotenoids [2], as well as agaran-type polysaccharides [3]. Unexpectedly, a high amount of carbohydrates was also found in a methanol-water (8:2) extract from *V. lanosa*, rich in sulfated bromophenols [4]. Aim of the current study was, therefore, to explore the chemical composition of these carbohydrates. Poly- and oligosaccharides were obtained from the water soluble partition of the crude aqueous methanolic extract by ethanol precipitation. The precipitate was analyzed by gel permeation chromatography, ¹³C-NMR and GC-QTOF-MS after hydrolysis. Dialyzed fractions were mainly composed of polysaccharides with molecular weights of 200 to 250 kDa. In line with previous findings, they mainly represented sulfated agar-type galactans (→[3]-β-d-Galp-(1→4)-α-l-Galp/-3,6-anhydro-Galp-(1→n), containing glucose, xylose and arabinose as substituents. High amounts of glucose additionally suggested the presence of floridean starch. Interestingly, the monosaccharide composition varied significantly among samples collected at different time points. Also, fucose was tentatively identified by GC-MS as a further substituent. Apart from polysaccharides, fractions of lower molecular weight (≤5 kDa) were found to contain mannose as the predominant monomer, although mannans have rarely been described in the algal order Ceramiales.

In summary, the current study provides new insights into the carbohydrate composition of the popular red alga *V. lanosa*.

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P124 Structure revision and NMR data characterization of natural 3β,11-dihydroxy-9,11-secoosteroids bearing a 5,6-epoxy group based on a single-crystal X-ray diffraction analysis

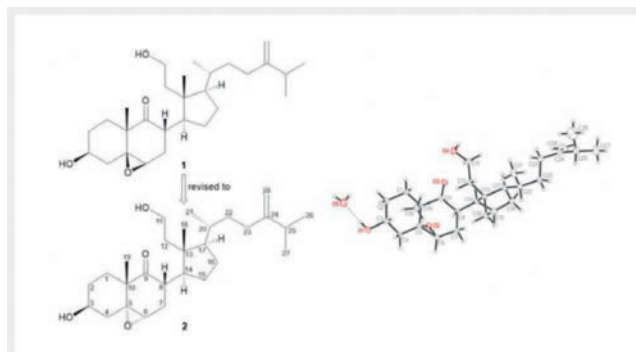
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A comprehensive structural analysis was conducted on a natural product previously identified as 3β,11-dihydroxy-5β,6β-epoxy-24-methylene-9,11-secocholestan-9-one (**1**) [1], which was reisolated from the Taiwanese octocoral *Sinularia lochmodes*. Comparative analysis of 1D and 2D NMR data, supported by X-ray crystallography, led to the revision of this compound's structure from **1** to its stereoisomer, 3β,11-dihydroxy-5α,6α-epoxy-24-methylene-9,11-secocholestan-9-one (**2**) (► Fig. 1). Based on these findings, the structures of

several previously reported 3β,11-dihydroxy-9-oxo-9,11-secoosteroids containing a 5,6-epoxy group should be re-evaluated by comparison with the NMR data of **2** [2–4]. Furthermore, this study establishes a correlation between the ¹H/¹³C NMR spectroscopic data and the absolute configurations of 9,11-secoosteroids bearing either a 5α,6α- or 5β,6β-epoxy group. The corrected molecular structure **2**, along with structurally related compounds and accurately determined ¹H and ¹³C NMR spectroscopic data confirmed in this study, could facilitate precise chemical structure assignments, enhance bioactivity predictions via molecular docking, and support reliable structure-activity relationship (SAR) analyses for future pharmaceutical research.



► **Fig. 1** Reported structure, 3β,11-dihydroxy-5β,6β-epoxy-24-methylene-9,11-secocholestan-9-one (**1**) and revised structure **2** (3β,11-dihydroxy-5α,6α-epoxy-24-methylene-9,11-secocholestan-9-one) and a computer-generated ORTEP plot revealing the absolute structure of 3β,11-dihydroxy-5α,6α-epoxy-24-methylene-9,11-secocholestan-9-one (**2**).

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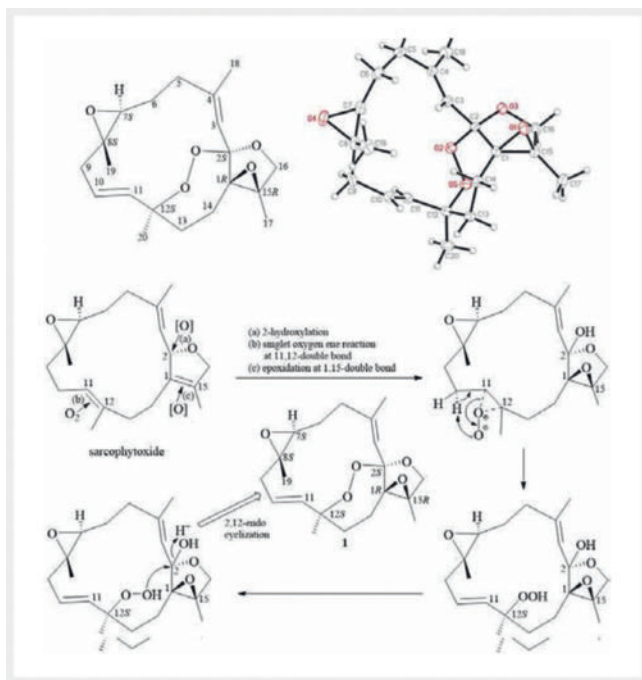
P125 Sarcophytonin H: a novel endoperoxide-containing dihydrofuranocembranoid from an octocoral *Sarcophyton* species

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Chemical composition screening of an octocoral identified as *Sarcophyton* species led to the isolation of a novel dihydrofuranocembranoid, sarcophytonin H (**1**), characterized by an endoperoxide moiety. The structure of **1** was determined through spectroscopic analysis and single-crystal X-ray diffraction (SC-XRD) analysis (► Fig. 1). This is the first reported instance of a 14-membered carbocyclic cembranoid analogue containing an endoperoxide group bridging C-2 and C-12. Additionally, the absolute configuration of (24S)-24-methylcholestan-3β,5α,6β,25-tetrol 25-monoacetate (**2**), also obtained in this study, was reported for the first time using SC-XRD. Dihydrofuranocembranoid **1** exhibited activity in enhancing alkaline phosphate (ALP) activity.



► **Fig. 1** Structure of sarcophytonin H (**1**), the computer-generated Oak Ridge Thermal Ellipsoid Plot (ORTEP) diagram of compound **1**, and a proposed biogenetic pathway for the sarcophytonin H skeleton.

P126 Metabolomics reveal pathways associated with neuroprotective effect of *Aquilaria crassna* extract fraction in 6-OHDA-induced mitochondrial dysfunction in SH-SY5Y cells

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Aquilaria crassna (AC) is widely used in traditional medicine for the treatment of various ailments. The current findings exerted the neuroprotective effect of AC fraction on 6-OHDA-induced SH-SY5Y models of Parkinson's disease (PD). The mitochondrial dysfunction in neurons is a characteristic of neurodegenerative diseases, particularly PD. The AC fraction could prevent the 6-OHDA-induced mitochondrial dysfunction through the inhibition of mitochondrial depolarization and the phosphorylation of α -synuclein [1, 2]. Confirmed by the metabolomics approach, the most potential metabolite associated with the mitochondrial dysfunction, including isoleucine, were significantly altered among different treatment groups. Additionally, the expression of isoleucine in each sample was confirmed using a targeted metabolomics approach. Disturbances in the metabolism of these metabolites afterwards develop PD characteristics. Pathway analysis showed that valine, leucine, and isoleucine biosynthesis was the prominent pathway. In conclusion, AC fraction may be utilized for the prevention of mitochondrial dysfunction in neurons and developed as

an alternative medicine for the treatment of neurodegenerative diseases, especially PD.

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P127 Advanced technology for purification of *N*-alkylamides from *Acmella oleracea* (L.) R.K. Jansen and development of innovative, safe and eco-friendly larvicidal extracts

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The recent increase of insecticide resistance highlighted an urgent need for innovative products from natural sources. The aim is to reduce the excessive use of synthetic pesticides boosting resistance development in mosquitoes and causing serious threats to human health and environment [1]. A promising candidate is *Acmella oleracea* (L.) R.K. Jansen (Asteraceae). Its known insecticidal activity depends on the presence of *N*-alkylamides, mainly represented by spilanthal. The research on this compound evidenced the necessity for innovative and solvent-free extraction protocols that could substitute the traditional techniques. In this work, an eco-friendly *N*-alkylamides-enriched fraction (AEF) from *A. oleracea* was obtained by combining for the first time supercritical fluid extraction (SFE) and wiped-film short path molecular (WSM) distillation techniques. As outlined by the HPLC-DAD analysis, this fraction was characterized by a significantly higher content of spilanthal and *N*-alkylamides than the SFE. Then, larvicidal assays were performed against 3rd instar larvae of *Aedes albopictus* Skuse, *Anopheles stephensi* Liston, and *An. gambiae* Giles to evaluate the activity of SFE, AEF, and pure spilanthal. They showed that the insecticidal activity of AEF is comparable to that of spilanthal and higher than that of SFE. Moreover, the dermal safety of these products was confirmed on human keratinocytes (HaCaT) [2]. Thanks to this study, new plant-derived pesticides could be manufactured through the utilization of WSM distillation to obtain a completely green product. In addition, it continues to pave the way for the development of effective, eco-friendly and safe botanical insecticides starting from active ingredients found in plant extracts.

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P129 An *in vitro* study of South African *Ziziphus*' antiparasmodial and antigonococcal bioactivity

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For centuries, *Ziziphus* species have been used in African traditional medicine as remedies for human ailments. In this study, South Africa's indigenous *Ziziphus* species (*Z. mucronata*, *Z. rivularis*, and *Z. zeyheriana*) are evaluated for their antiplasmodial and antigonococcal phytoconstituents. The plant parts were air-dried, and sequentially extracted using *n*-hexane, dichloromethane, ethyl acetate, ethyl acetate:methanol (1:1), and methanol. Forty-eight extracts were obtained and subjected to antiplasmodial and anti-gonococcal bioassays. Further NMR-based metabolomics to predict bioactivity in unknown samples and identify active phytoconstituent classes was conducted. The PCA and OPLS-DA analysis was applied to reduce dimensionality and contribution plots were generated to observe discrimination according to observed bioactivity. Of the 48 assayed extracts, 31 and 54 % were inactive against malaria and gonorrhea, respectively. For malaria, 13 and 23 % corresponded to high and moderate activity respectively. For gonorrhea, 12.5 and 33.3 % corresponded to extracts with high and moderate activity, respectively. *Ziziphus rivularis* leaves recorded the highest antigonococcal activity (MIC < 1 mg/mL) and *Z. zeyheriana* roots displayed the best antiplasmodial activity (IC₅₀ < 5 µg/mL). NMR-based metabolomics predicted antiplasmodial and antigonococcal activity with OPLS-DA displaying P-values < 0.05. Aliphatic, allylic, methyl ketone, and carboxylic-based phytoconstituents were tentatively attributed to the observed antiplasmodial activity. Antigonococcal compound classes include aliphatic, alcohol, aldehyde, and carboxylic acid-based constituents. The study demonstrates the potential of bioprospecting *Ziziphus* species for antiplasmodial and antigonococcal bioactivity. It marks a significant milestone as the first to report *in vitro* bioactivity in *Ziziphus rivularis* and *Ziziphus zeyheriana* [1, 2].

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P130 Biological activity and cytotoxicity studies on *Hypericum montbretii* Spach (Hypericaceae)

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The genus *Hypericum* Tourn. ex L. is represented by 96 species and 116 taxa in Türkiye and is an important genus with an endemism rate of 40 % [1]. In this study, antioxidant, antimicrobial, and cytotoxicity activities of methanol and ethyl acetate extracts prepared from aerial parts of *Hypericum montbretii* Spach were investigated.

MIC values of extracts were analyzed by microdilution method. Ethyl acetate extracts were found to be effective against *Pseudomonas aeruginosa*, *Staphylococcus aureus* standard bacteria at a concentration of 3 µg/mL, *Bacillus subtilis*, *Escherichia coli* bacteria at 7 µg/mL and *Candida albicans* with *Candida krusei* yeasts at a concentration of 930 µg/mL. Methanol extracts were effective against *Pseudomonas aeruginosa* and *Bacillus subtilis* at a concentration of 541 µg/mL, *Escherichia coli*, and *Staphylococcus aureus* at a concentration of 1083 µg/mL, and also *Candida albicans* with *Candida krusei* yeasts at a concentration of 270 µg/mL.

The cytotoxic activity of extracts was assessed using the MTT assay and NIH3T3 mouse embryonic fibroblast cells were used for the cytotoxicity test. The IC₅₀ value of the methanol extract (135.4 ± 0.21 µg/mL) was found to be higher compared to ethyl acetate extract (> 500 µg/mL).

Antioxidant activity was evaluated using the DPPH method. Methanol extract showed 46 % inhibition, while ethyl acetate showed 23 % inhibition. Total phenol content was determined using the Folin-Ciocalteu method, revealing that the methanol extract (99.03 ± 0.086 mg GAE/g) contained a higher phenolic content compared to the ethyl acetate extract (65.00 ± 0.079 mg GAE/g).

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P131 Saponins and phenolic compounds in *Quillaja brasiliensis* leaves: In situ histochemical localization and anthelmintic activity

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Quillaja brasiliensis (Qb), commonly known as the “soap tree”, is a saponin-producing tree native to Uruguay, Brazil, Argentina, and Paraguay. This study draws inspiration from the reported biological activities of its congener, *Quillaja saponaria* (Qs), which has saponins and phenolic compounds that exhibit significant immunoadjuvant, molluscicidal, antiparasitic, and antiviral properties [1, 2]. The objectives of this work were to: a) histolocalize the accumulation sites of saponins and phenolic compounds in Qb leaves; b) characterize the main phenolic compounds; and c) determine the *in vitro* anthelmintic activity of extracts enriched in saponins and phenolic compounds.

Triterpenic saponins were found in epidermal cells and the palisade parenchyma. Using ferric chloride reagent, phenolic compounds were detected in the mesophyll and collenchyma, while vanillin-HCl revealed the presence of tannins in the palisade parenchyma and collenchyma.

Through LC-MS/MS analysis, 31 phenolic compounds were identified, including hydroxycinnamic acid derivatives, hydroxybenzoic acid, flavonols, flavones, flavanones, flavan-3-ols, and condensed tannins. One compound was isolated, and its structure was elucidated by MS and NMR, revealing it to be 19-O-β-d-glucopyranoside of 16-hydroxylambertic acid.

The inhibitory activity of Qb leaf extracts enriched in phenolic compounds and saponins was evaluated against gastrointestinal nematodes in naturally infected cattle. The anthelmintic activity of the isolated compound was also tested. This study represents the first report on the histolocalization of saponins and phenolic compounds in the leaves of Qb. Some of the phenolic compounds identified in Qs were also found in Qb [3]. Saponins from Qb leaves exhibit higher anthelmintic activity than phenolic compounds.

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P132 Plant-derived active pharmaceutical ingredients target macrophage polarization and phagocytosis and reprogram systemic metabolism toward anti-melanoma metastases

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DOI 10.1055/s-0045-1815037

Melanoma is the most aggressive form of skin cancer, with approximately 50 % of cases harboring BRAF mutations, contributing to high metastatic potential and poor prognosis. BRAF inhibitors, such as vemurafenib (PLX4032), are used in late-stage melanoma; however, acquired resistance and adverse effects limit their efficacy. This study identified active pharmaceutical ingredients (API) from a medicinal Asteraceae plant (designated BC) using a bioactivity-guided approach which shows potent anti-metastatic effects on melanoma lung metastasis. The BC_API significantly inhibited the epithelial-mesenchymal transition phenotype, reduced cancer stem-like characteristics, and induced apoptosis in metastatic melanoma cells. In a high-propensity lung metastatic A375LMeIFAg/Luc xenograft mouse model, treatment with PLX4032 (50 mg/kg) and BC_API (20 mg/kg) suppressed lung and liver metastases and extended animal survival to 40 and 34 days, respectively, compared to 26 days in control mice. In the lung tumor microenvironment, M2 macrophages were detected abundantly in tumor nests and frontal tumor regions which were polarized into M1 macrophages after API treatment. *In vitro* studies further confirmed that the BC_API promoted M1 macrophage polarization and its phagocytotic ability. GC/MS-based metabolomics and KEGG-database pathway analysis showed that BC_API affected primary metabolism in tumor-bearing mice, such as unsaturated fatty acid metabolism or biosynthesis and arginine biosynthesis that might be associated with melanoma invasion/metastasis, M1 macrophage polarization, and oxylipin metabolism, contributing to the anti-tumor metastasis effect of the API. This study highlights the great potential of a phytoAPI as botanical drug candidate for metastatic melanoma therapy [1, 2].

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P133 Isoflavone constituents of *Iris florentina* L. roots and their inhibitory effects on the formation of advanced glycation end products

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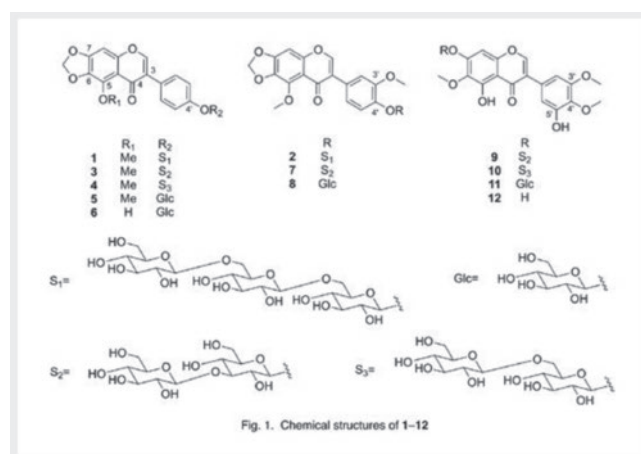
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DOI 10.1055/s-0045-1815038

The genus *Iris* (Iridaceae) comprises approximately 150 species, among which *Iris florentina* L. is distributed along the Mediterranean coast of Europe. The dried roots of this plant, called Orris roots, have been traditionally used as a perfume ingredient and a fixative in aromatherapy. Although a few norisoprenoids, xanthone glucosides, and flavone glucosides have been isolated from *I. florentina* [1], there has been no systematic phytochemical study of its secondary metabolites. Therefore, we conducted a phytochemical examination of *I. florentina* and evaluated the inhibitory activity of the isolated compounds on the formation of advanced glycation end products (AGEs).

The roots of *I. florentina* were extracted with MeOH, and the concentrated MeOH extract was passed through a Diaion HP-20 column, successively eluted with 30 % MeOH, 50 % MeOH, MeOH, EtOH, and EtOAc. The MeOH eluate frac-

tion was subjected to column chromatography on silica gel and octadecylsilanized silica gel, yielding compounds 1–12 (► Fig. 1).



► Fig. 1 Chemical structures for 1–12

The structures of the new isoflavonoid glycosides (1 and 2) were determined by spectroscopic studies, including 2D NMR spectral data, and chromatographic and spectroscopic analyses of the hydrolyzed products.

AGEs are formed either through non-enzymatic glycation and oxidation of proteins and reducing sugars, or as intermediates in carbohydrate metabolism. The accumulation of AGEs in the skin, blood vessel walls, and bones is closely related to aging. Among the isolated isoflavonoids, 6 and 10–12 inhibited the formation of AGEs, with IC₅₀ values of 8.0, 8.8, 6.5, and 2.1 mM, respectively, whereas aminoguanidine, used as a positive control, exhibited an IC₅₀ value of 15.6 mM.

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P134 Steroidal components of the whole plants of *Helleborus niger* L. and their cytotoxicity

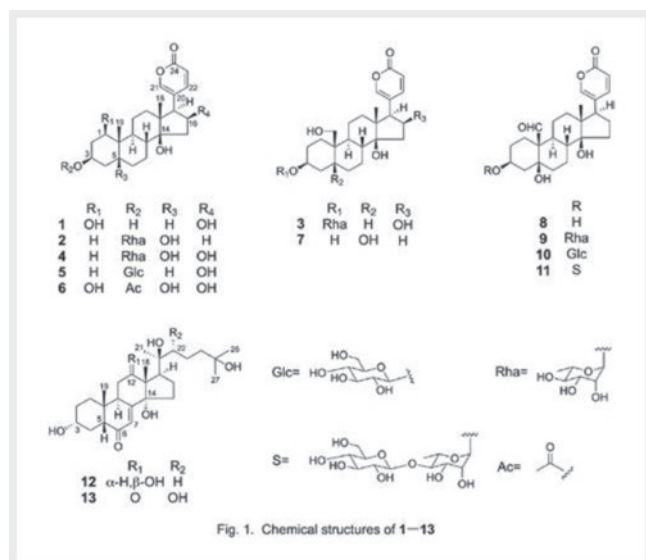
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The genus *Helleborus* (Ranunculaceae) includes about 20 species, most of which are cultivated for ornamental purposes. Among them, *Helleborus niger* L., commonly known as Christmas rose, is native to the alpine regions of southern and central Europe. Our previous phytochemical studies on *H. foetidus* L. and *H. lividus* Aiton ex Curtis led to the isolation of diverse steroidal compounds, some of which exhibited potent cytotoxicity in cultured tumor cells [1, 2]. Therefore, we conducted a phytochemical examination of *H. niger* and evaluated the cytotoxicity of the isolated compounds.

The whole plants of *H. niger* were extracted with MeOH. The concentrated MeOH extract was passed through a Diaion HP-20 column, successively eluted with 30 % MeOH, 50 % MeOH, MeOH, EtOH, and EtOAc. The MeOH eluate fraction was subjected to column chromatography on silica gel and octadecylsilanized silica gel, giving compounds 1–13 (► Fig. 1).



► Fig. 1 Chemical structures for 1–13

The structures of the new bufadienolide (**1**), bufadienolide rhamnosides (**2** and **3**), and ecdysteroids (**12** and **13**) were determined by spectroscopic studies, including 2D NMR spectral data, and chromatographic and spectroscopic analyses of the hydrolyzed products.

Compounds **1–12** showed cytotoxicity against HL-60 human leukemia cells, A549 human lung adenocarcinoma cells, SBC-3 human small-cell lung cancer cells, with IC_{50} values ranging from 0.0016–6.1 μ M. In contrast, **13** did not show cytotoxicity.

Compound **2** exhibited potent cell proliferation inhibitory activity against SBC-3 cells after 24–48 h of treatment and induced apoptosis in SBC-3 cells via an intrinsic pathway after 72 h of treatment.

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P135 Phytochemical composition and biological activity of the fractions obtained from the methanolic extract of *Eleutherococcus senticosus* roots

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Eleutherococcus senticosus (Rupr. & Maxim.) Maxim. (ES) is a plant used in traditional medicine for its adaptogenic, antioxidant, and anti-inflammatory properties. The main active compounds found in the roots include lignans (syringaresinol, sesamin), phenolic acids (chlorogenic, caffeic), coumarins (isofraxidin), and flavonoids (kaempferol, quercetin, rutin). The aim of this study was to determine the chemical composition and biological activity of the four fractions of ES, with particular focus on the potential therapeutic applications in the treatment of chronic diseases such as diabetes, neurodegenerative diseases, and cancer [1, 2].

The root extract of *E. senticosus* exhibits strong antioxidant properties, primarily attributed to its high content of phenolic acids, especially chlorogenic acid.

The fractions differ in activity, with the ethyl acetate fraction showing the highest capacity for free radical scavenging and metal ion reduction. Anti-enzymatic activity was also observed, particularly in the inhibition of hyaluronidase and α -amylase, suggesting potential applications in anti-inflammatory and antidiabetic therapies. The chemical composition of the extract includes a wide range of polyphenolic compounds, notably derivatives of benzoic and cinnamic acids, which are likely responsible for the observed biological effects.

The studies confirmed the strong antioxidant, anti-inflammatory, and anti-enzymatic properties of *E. senticosus* fractions. The results mean that the ethyl acetate fraction has a potential to be used in the treatment of diseases associated with oxidative stress and inflammation. Further research into the mechanisms of action and clinical applications is necessary.

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P138 Cosmeceutical activity of *Rhabdocalon lavanduloides* essential oil and ethylacetate extracts

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DOI 10.1055/s-0045-1815042

Popularly known as lavanda-do-Brasil, *Rhabdocalon lavanduloides* (Benth.) Epling (Lamiaceae), is an endemic species found in Brazil in the Atlantic Forest and Cerrado biomes. In this work, essential oils from dry leaves (RLOE-1), and dry stem (RLOE-2), Soxhlet ethylacetate extracts from dry flowers (RLFI), dry leaves (RLF) and dry stem (RLC) of *R. lavanduloides* were tested for their cosmeceutical activity using anti-elastase, anti-collagenase, and anti-tyrosinase assay and the results were expressed as the respective IC_{50} values. The major constituent of the essential oil, the terpenoid 2-methoxy-carvacrol (RL2M), isolated from the essential oil of dry leaves was also subjected to the analysis. The highest anti-tyrosinase activity was displayed by RLFI ($131.6 \pm 5.7 \mu$ g/mL), however, the tyrosinase inhibitory activity of the extract was moderate, and not reaching the activity of the standard, kojic acid ($0.35 \pm 0.01 \mu$ g/mL) (ANOVA followed by Dunnett's post-hoc test, $p < 0.05$). On the other hand, the samples demonstrated excellent anti-collagenase activity that did not statistically differ from the activity of the employed standard, gallic acid ($114.2 \pm 12.7 \mu$ g/mL). The most active in this assay were RLOE-1 ($96.3 \pm 2.8 \mu$ g/mL) and RLOE-2 ($110.7 \pm 8.3 \mu$ g/mL), as well as RLF ($92.2 \pm 3.4 \mu$ g/mL). Similarly, RLF ($414.1 \pm 3.5 \mu$ g/mL) RLFI ($498.0 \pm 54.3 \mu$ g/mL) and RLC ($378.1 \pm 42.3 \mu$ g/mL), displayed the anti-elastase activity equal to the activity of ursolic acid ($313.6 \pm 38.2 \mu$ g/mL). Overall, the essential oils and the extracts of *Rhabdocalon lavanduloides* displayed an excellent activity in the performed assays granting further research on this species. However, it seems that RL2M is not the constituent responsible for the observed activity.

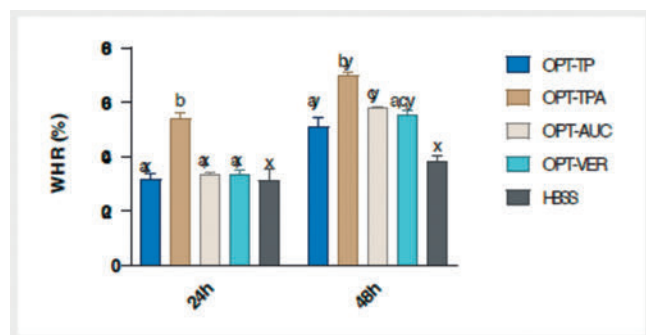
P139 Phytochemical analysis and wound healing potential of hydroxypropyl- β -cyclodextrin glycerol based *Plantago major* L. extracts

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Plantago major L. is one of the species from the plantain family Plantaginaceae which is widely spread over the world. The aerial parts of *P. major* contain active ingredients such as flavonoids, polysaccharides, terpenoids, lipids, iridoid glycosides, and caffeic acid derivatives. A range of biological activities has been found from plant extracts including wound healing activity, anti-inflammatory, analgesic, antioxidant, antibiotic, immune modulating [1]. In this work, the extracts of *P. major* were prepared with biocompatible solvents such as: water, glycerol and lactic acid with addition of 2-hydroxypropyl- β -cyclodextrin by using the ultrasound assisted extraction. The content of bioactive compounds (aucubin and verbascoside) in the extracts was determined by using HPLC-DAD method. According to the results of HPLC analysis, four extracts were prepared: the extract with the highest concentration of total polyphenols (PM-TP; 2158 μ g/mL), total phenolic acid (PM-TPA, 1483.15 μ g/mL), aucubin (PM-AUC, 999.503 μ g/mL), and verbascoside (PM-VER, 126 μ g/mL). Cell viability was assessed by using the MTT assay in extract concentration interval between 1.25-250 μ L extract/mL. The wound-healing properties were performed by *in vitro* "scratch test" using HaCaT cells [2]. The results of MTT assay have shown cell viability exceeding 90 % at lower concentrations (1.25-12.5 μ L extract/mL). Furthermore, the extracts also demonstrated the capacity to enhance wound healing in the *in vitro* scratch wound model, especially extract PM-TPA which had above 70 % of scratch surface area recovered (► Fig. 1). The observed biocompatibility of *P. major* extracts prepared by using environmentally friendly solvents makes them promising ingredients as cosmeceuticals.



► Fig. 1 Capacity of *Plantago major* extracts to enhance wound healing in the *in vitro* scratch wound model.

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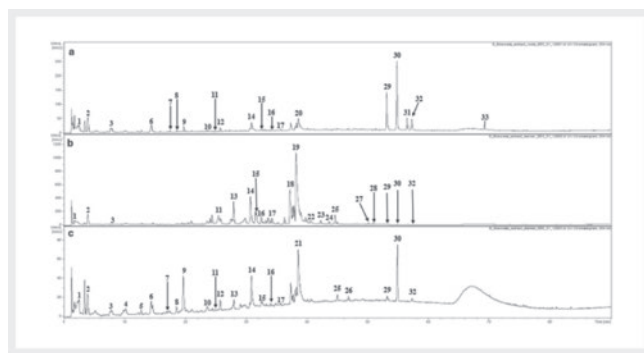
P142 Antioxidant activity and phytochemical profile of leaf, stem bark, and root bark aqueous extracts of *Boswellia dalzielii* Hutch: an *in vitro* and UHPLC-DAD-MS studies

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This presentation will provide a comprehensive analysis of the antioxidant activity and phytochemical profile of aqueous extracts from the leaf, stem bark, and root bark of *Boswellia dalzielii* Hutch. (*B. dalzielii*), based on *in vitro* studies and UHPLC-DAD-MS analysis. *B. dalzielii* (Burseraceae) is one of those plants used by traditional healers to treat several disease conditions, including mental illness, bowel and skin infections, and wounds. Previous research has documented the antibacterial, antiulcer, antispasmodic, hepatoprotective, and hypoglycemic activities [1, 2] and behavioural effects [3] of *B. dalzielii*. Therefore, the present study aimed to evaluate the antioxidant activities and comprehensive phytochemical analysis of aqueous extracts from the leaf, stem bark, and root bark of *B. dalzielii* using various *in vitro* assays and HPLC-DAD-MS analysis, providing valuable insights into its therapeutic usage and promoting further research into its medicinal properties. The result demonstrates that different parts of *B. dalzielii* exhibit dose-dependent free radical scavenging abilities. Among the plant parts assessed, the leaf extract displayed the highest antioxidant activity across most parameters, suggesting a higher concentration or greater efficacy of antioxidant compounds. The root bark extract also showed considerable scavenging activity, likely due to the effectiveness or specific composition of its bioactive constituents. The phytochemical analysis revealed several phytoconstituents, including phenolic acids, flavonoids, and stilbenoids, with a stilbenoid detected in all parts of the plant, although some compounds remain undefined (► Fig. 1). These findings suggest that *B. dalzielii* could be a valuable natural source of antioxidants, particularly in addressing disorders linked to oxidative stress. Ongoing investigations in our laboratory aim to isolate and identify the unidentified compounds present in the plant extracts.



► Fig. 1 UV chromatograms of root, leaf, and stem bark extract at 254 nm.

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P143 Preserving traditional medicinal plant use: A review of indigenous wisdom in Nigeria

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DOI 10.1055/s-0045-1815045

Indigenous knowledge systems form the backbone of traditional healthcare practices across Nigeria, reflecting a deep-rooted cultural relationship between people and their natural environment [1, 2]. This review examines traditional medicinal plant use across Nigeria, synthesising insights from 3,536 academic sources, of which 79 were analysed in detail. The studies document 962 plant species from 144 botanical families, illustrating a rich ethnobotanical heritage. Frequently cited species included *Carica papaya* L. and *Vernonia amygdalina* Delile, each mentioned 42 times. The Fabaceae family was particularly prominent with 127 species recorded. Trees made up 55 % of the documented species, highlighting the ecological importance of forests in traditional healing systems. The review identified 78 health conditions treated with these botanicals, with the most common being malaria (33.51 %), cancer (22.23 %), and cough (21.33 %). Leaves (41 %) were the most utilized part, followed by bark (21 %), roots (15 %), fruits (7 %), and seeds (7 %). Decoction was the primary preparation method, along with infusion, powdering, and maceration. Although oral administration was the most common, topical applications for skin ailments were also prevalent. By cataloguing and analysing this vast body of ethnobotanical knowledge, the review highlights the urgent need to preserve the indigenous medicinal heritage of Nigeria. The findings reaffirm the significance of traditional medicine in contemporary health systems and advocate for the documentation, conservation, and respectful integration of indigenous knowledge into wider healthcare and research frameworks.

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P144 An appraisal on the use of medicinal plants against sexually transmitted infections in sub-Saharan Africa

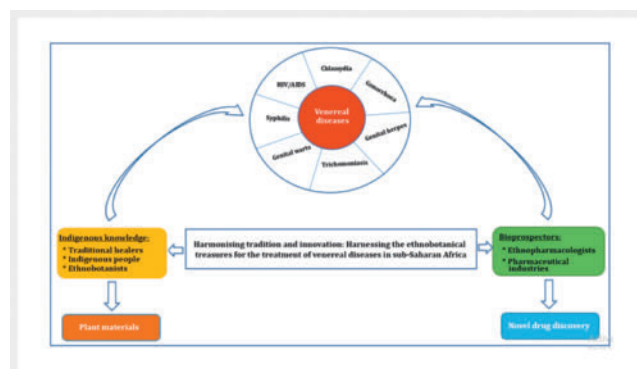
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Sub-Saharan Africa has one of the highest burdens of venereal diseases (VDs) globally, presenting a critical public health challenge [1], for which medicinal plants offer potential therapeutic solutions [2]. This review critically evaluates the use of plants for treating VDs across the region. Adhering to the PRISMA protocol [3], we assessed literature focusing on studies published from 2008 to May 16, 2024. Of the 131 studies reviewed, 20 ethnobotanical reports were identified, with Nigeria and South Africa contributing the most (25 % each). We documented 445 anti-venereal plants spanning 99 families, sourced from over 872 Indigenous knowledge holders. Notable plants included *Cassia abbreviate* Oliv., *Ziziphus mucronate* Willd., *Ximenia caffra* Sond., *Catharanthus roseus* (L.) G.Don, and *Terminalia prunioides* M.A. Lawson. In the analysis of 98 plants based on high ethnobotanical index, Fabaceae was the most represented (15.8 % with 16 species), followed by Cucurbitaceae and Solanaceae (5.9 % each). Roots (41.5 %) and leaves (26.3 %) were predominantly utilised, highlighting a potential threat from destructive harvesting practices [4]. Decoctions (36.7 %) and infusions (12.2 %) were common preparation methods, and oral administration (72.9 %) was predominant. We consolidated valuable Indigenous knowledge, crucial for ethnopharmacological research and potential drug discovery (► Fig. 1). While many species are utilised, the emphasis on root harvesting and the existence of threatened species indicate the need for sustainable harvesting practices, further research, stringent regulations, and cross-country collaborations to conserve and utilise these medicinal resources responsibly. Documenting this knowledge is essential to preserve cultural heritage and guide the development of effective and culturally sensitive VD treatments.



► Fig. 1 Overview on the ethnobotanical documentation and conservation of medicinal plants used against sexually transmitted infections in sub-Saharan Africa.

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P145 Natural triterpenoid compounds modulating AMPK-mediated energy metabolism in *Caenorhabditis elegans* model of obesity

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Triterpenoids possess promising anti-obesogenic potential modulating multiple key molecular mechanisms, which is attributed to their structural diversity. By targeting essential signaling pathways such as the adenosine monophosphate protein kinase (AMPK) and sirtuin (SIRT) pathway, triterpenoids may represent an effective strategy for obesity management [1]. Moreover, these compounds are known to counteract systemic low-grade inflammation, which is a critical obesity-related metabolic dysfunction [2].

Within the current study we have evaluated the anti-obesogenic activity of selected triterpenoids (gypenoside XLIX, pulchrenoside C, momordin Ic) as they have been scarcely investigated in the context of obesity and metabolic diseases. Therefore, we monitored key body parameters (as mean width, length, and surface area), and evaluated the effect on the lipid accumulation in a *Caenorhabditis elegans* obesity model. The selected compounds were applied at a treatment concentration of 100 µM in both wild-type and MIR13 strains. Orlistat was utilized as a control treatment, being an approved anti-obesity medication.

Our findings demonstrated that the selected triterpenoids effectively modulate lipid metabolism in nematodes supplemented with 2% glucose.

Collectively, the data suggest a rationale for further investigation of gypenoside XLIX, pulchrenoside C, momordin Ic as modulators of glucose-induced lipid accumulation and identification of specific molecular mechanisms of action in the context of obesity and metabolic disorders.

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P146 Untargeted metabolite profiling of syrup rose ecotypes cultivated in the Alta Valle Scrivia (Liguria, Italy)

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“Valle Scrivia rose syrup” (VSrs) is an ancient Italian recipe, used both as a drink and to relieve coughs and bronchitis [1], produced by infusing rose petals from ancient plants cultivated in Liguria, and awarded as Slow Food Presidium. Three rose ecotypes are mainly used, locally known as “muscosa”, a mutation of *R. centifolia*, a presumed hybrid between a *R. gallica* and *R. damascena*, “rugosa”,

attributed to the group derived from *R. rugosa*, and “damask” (*R. x damascena*). Aim of the project was the chemical profiling of these ecotypes, collected from different farms of the valley, through LC-MS and NMR metabolomic based approach to contribute to the characterization of these ecotypes and to the valorization of local products. The ¹H NMR profiling revealed the presence of amino acids, such as arginine and asparagine, organic acids as quinic acid. Among sugars, detectable in the middle part of the ¹H NMR spectrum, glucose was the most abundant. Several aromatic protons, belonging to phenolic compounds, were also detected. LC-HRESIMS/MS analysis led to the annotation of several flavonoids, especially quercetin and kaempferol derivatives, and anthocyanins, such as cyanidin, pelargonidin, and delphinidin hexosides. Moreover, among gallotannins, tri-O-galloylhexose, tetra-O-galloylhexose and penta-O-galloylhexose were identified [2]. Data from NMR and LC-MS analysis were subjected to multivariate analysis (PCA, HC, SOM). Results showed two clusters, one made up of all “muscosa” and “rugosa” samples, mainly characterized by quercetin O-hexoside-dehydroxyhexoside and delphinidin O-hexoside, and one of all “damask” samples, mainly characterized by cyanidin O-hexoside.

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P147 *Albizia julibrissin* leaf extract induces EGFR-targeted apoptosis in human pancreatic cancer

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DOI 10.1055/s-0045-1815049

Pancreatic cancer, known for late-stage diagnosis, rapid progression, and poor prognosis, remains a critical global health issue as the seventh leading cause of cancer-related death. Current treatments encounter significant limitations due to drug resistance and toxicity, highlighting the urgent need for safer, effective therapies. This study explored the anti-cancer effects of *Albizia julibrissin* leaf extract (AJLE), prepared via hot water extraction, utilizing both *in vitro* and *in vivo* models. *In vitro* assays, including WST-1 cell viability, colony formation, flow cytometry-based cell cycle analysis, and western blotting for apoptosis markers, revealed AJLE significantly inhibited pancreatic cancer cell proliferation, suppressed colony formation, and induced cell cycle arrest at the sub-G1 phase. Apoptosis was confirmed by increased cleaved PARP and caspase-3 levels and characteristic nuclear changes via fluorescence microscopy. Mechanistically, AJLE inhibited MAPK and PI3K/AKT signaling pathways and notably increased EGFR expression, suggesting EGFR-mediated apoptosis as an important anti-cancer mechanism. *In vivo* xenograft mouse studies demonstrated a dose-dependent decrease in tumor growth without significant liver or kidney toxicity, as validated through histopathological and serum analyses. Elevated cleaved caspase-3 levels in tumors from AJLE-treated mice further supported apoptosis induction. These findings highlight AJLE's potential as an effective natural anti-cancer agent against pancreatic cancer, presenting a targeted, less toxic alternative or complementary therapy to conventional treatments (RS-2024-00405028; RS-2023-00270936) [1, 2].

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P148 Traditional knowledge, phytochemical screening, antibacterial activity and cream formulation of *Teucrium polium* L. hydromethanolic extract.

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DOI 10.1055/s-0045-1815050

Teucrium polium L. (Lamiaceae) is popularly known in traditional Algerian medicine as «khayata» for its healing therapeutic properties [1, 2] (► **Fig. 1**).

The aim of the present study was to investigate traditional uses, and the qualitative content of phytochemical compounds and the antibacterial activity of *Teucrium polium* L. aerial parts hydromethanolic extracts [AP_HME]. In addition, a cream was formulated with 5 % of HME, the stability and skin tolerance were investigated at 40 °C for two months.



► **Fig. 1** Macroscopical identification of *Teucrium polium* L. collected in M'sila region.

The [AP_HME] showed a yield of 13.34 % dr/dw. The results showed that 48.0 % of respondents use the aerial parts of *T. polium* for the treatment of gastrointestinal disorders, while 34.0 % use it for wound healing. Phytochemical screening has demonstrated several bioactive compounds including polyphenols, flavonoids, catechic tannins, cardiotonic heterosides, and coumarins. The foam index was also determined with the highest value of 667 in the aerial parts of the plant at its flowering stage.

The antibacterial activity was significant against *Serratia* sp., *Klebsiella pneumoniae*, and *Staphylococcus aureus* MRSA 1 [8.9–11.3 mm]. A 5 % w/w [AP_HME] was incorporated into a cream base including sweet almond oil, Span 60, Tween 20, cetostearyl alcohol, beeswax, and purified water. The stability was con-

firmed at 40 °C for two months, indicating suitability for dermatological applications. This framework validates traditional uses and suggests potential for modern pharmaceutical development. Future research should focus on identifying specific active compounds, assessing *in vivo* toxicity and conducting clinical trials to validate commercial applications.

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P149 Contemporary clinical practice, ethnobotanical and traditional evidence and pre-clinical research-alignment, limitations and opportunities

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DOI 10.1055/s-0045-1815051

Traditional herbal treatments, substantial part of traditional medicine across all traditional medicine systems, were prepared using whole herb(s) and/or parts of plants and in different forms, including teas, powders, and poultices. While traditional evidence and ethnobotanical research offer an avenue for exploring approaches to disease, they are often misinterpreted and used in a non-traditional, reductionist way to develop new pharmaceuticals and products by creating compound libraries using fractionation approaches with resulting compounds screened in simple pre-clinical biological assays. This bio-fractionation approach ignores the complexity and chemical synergy of medicinal plants and their traditional medicinal use resulting in expensive, lengthy and often ineffective drug development processes and criticism of medicinal plants effectiveness in the applicable disease state.

To address alignment between ethnobotanical research, traditional knowledge, contemporary clinical practice and pre-clinical research with the ultimate view of *natural product* development process that still aligns with the current staged research approach, we have developed a tradition-research nexus framework that considers not just medicinal plant complexity and their use but also extends pre-clinical research into using more complex *in vitro* models. Current presentation will describe the framework and its implementation into 2 different natural product projects currently underway in our laboratory (cancer and UTI biofilms) and will discuss limitations and opportunities of using this approach – framework flexibility, how do we account for plant complexity and possible synergy, what are intellectual property issues to consider, what are the implications for natural products industry and how does this benefit custodians of the wider botanical knowledge?

P150 Anti-rheumatoid arthritis and vascular endothelial improvement by *Derris scandens* Roxb. Benth. in adjuvant-induced arthritis in rats

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DOI 10.1055/s-0045-1815052

Derris scandens stem (DS), a Thai medicinal plant traditionally used to treat musculoskeletal pain, has shown some cardiovascular properties [1, 2]. However, its mechanisms and impact on cardiovascular disorders related to arthritis remain unexplored. We aimed to explore the impact of DS on endothelial dysfunction and traditional cardiovascular risk factors in a rat model of adjuvant-induced arthritis (AIA). From the onset of arthritis symptoms, DS (200 mg/kg/day, orally), methotrexate (1 mg/kg/week, subcutaneously), or their combination was administered for 21 days, with daily monitoring of body weight and arthritis scores. At the end of the treatment period, electrocardiography and blood pressure were measured in anesthetized rats. Endothelial function was evaluated in isolated pulmonary arteries (PA) and aortae through acetylcholine-induced relaxation. Plasma cytokine levels and leukocyte populations in peripheral blood and PA were measured. Direct DS-induced relaxation was also assessed in healthy rats. Results showed that direct exposure to DS induced greater relaxation in the PA (Emax = 90 %) than in the aorta (Emax = 38 %). Similar to methotrexate, DS significantly reduced arthritis severity compared to untreated AIA rats. In AIA rats, DS notably improved endothelial function in both the PA and aorta. Concurrently with the decrease in TNF- α and IL-1 β plasma levels, PA-infiltrated leukocyte populations, particularly neutrophils, were reduced after DS treatment. Co-administration with methotrexate diminished these vascular benefits. In summary, DS alleviates arthritis severity and enhances endothelial function in AIA rats by reducing leukocyte populations and cytokine levels. However, caution should be taken when combining DS with methotrexate.

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P151 Peppermint oil and caraway oil, the constituents of Menthacarin®, alleviate intestinal inflammation and ameliorate experimental colitis

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Mentha x piperita L. and *Carum carvi* L. essential oils are established remedies in gastrointestinal disorders and are the constituents of the proprietary combination Menthacarin® (90 mg peppermint oil WS® 1340 and 50 mg caraway oil WS® 1520). Menthacarin® is clinically used for the treatment of disorders of gut-brain interaction (DGBI) and *in vivo* studies demonstrated an attenuation of experimental colitis via anti-inflammatory effects [1]. In this study, we evaluated the therapeutic effects of the individual oils on the disease course and inflammatory mediators in an experimental colitis model.

Experimental colitis was induced by exposure of mice to 3 % dextran sulfate sodium administered via drinking water for 7 days. In parallel, animals were treated orally with vehicle, peppermint oil (20 mg/ kg) or caraway oil (10 mg/ kg). Body weight changes and colon length were determined, and pro-inflammatory markers associated with neutrophilic inflammation were assessed in colonic tissues by Bio-Plex Multiplex Immunoassay System.

Peppermint oil and caraway oil attenuated body weight loss accompanied by a significantly reduced shortening of the colon. Further, in colonic tissues with DSS-induced inflammation the concentrations of G-CSF and KC were significantly decreased by either treatment. Colonic levels of IL-6 and IL-17A were also notably lowered by peppermint oil and showed a tendency to decrease with caraway oil.

In conclusion, we show that both, peppermint oil and caraway oil, reduce severity of experimental colitis, which might be mediated by a reduced neutrophil-associated intestinal inflammation. This data indicates that both oils contribute to Menthacarin®'s attenuation of experimental colitis.

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P152 Urban ethnomedicine in Benguela, Angola – medicinal plants sold, uses and methods of preparation and administration

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Traditional medicine continues to be the first resort for many populations, both rural and urban, particularly in less favored countries [1]. In African city markets there are often areas selling medicinal products, mainly of plant origin, whose properties and uses are recognized by the resident populations [2, 3]. This study was developed in the market of the city of Benguela, in southern Angola, and aimed to document the diversity of medicinal plants sold, their respective uses and methods of preparation and administration, as well as the geographical origin of those plants. Fifty stalls selling medicinal plants were inventoried, where the sale of 78 medicinal products of plant origin were recorded. The common names in the local language (*Umbundo*) were recorded and 38 species have been identified so far.

A wide range of diseases and health conditions are treated with medicinal plants sold in the Benguela market. After applying the ICPC-2 categories we found that the illnesses most treated were D (digestive), A (general and unspecific) and Y (Male genital system).

The most commonly used plant parts are the roots (69 %). As for the preparation methods, the most common was infusion (55 %) and the most frequent method of administration was ingestion (65 %). The origin of the plants were mainly from the Benguela municipalities, but also from other provinces like Huambo. The plants sold are mostly purchased by sellers from specialized collectors. Our data reveal the importance of traditional medicine in an urban southern African context as the first resource easily accessible to the community.

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P153 From folk remedies to modern therapies: Ethnobotanical insights and bioactivity screening of Serra da Estrela (Portugal) medicinal plants

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Medicinal plants offer promising natural therapeutic solutions for managing diabetes and oxidative stress-related diseases, which are becoming increasingly prevalent worldwide. Portugal's Serra da Estrela Natural Park (SENP), in the country's central highlands, harbours one of its richest mountain floras; steep altitudinal and climatic gradients there stimulate the production of a wide range of secondary metabolites, making the park a valuable reservoir of potential bioactive compounds [1]. In this study, an ethnobotanical survey was conducted with local communities across 34 localities in five municipalities (Celorico da Beira, Covilhã, Guarda, Seia, and Gouveia), documenting traditional uses of dozens of plant species, predominantly from the Lamiaceae and Asteraceae families. Concurrently, 47 samples (leaves, flowers and fruits) were collected from SENP, processed into aqueous infusions, and evaluated for their antidiabetic, antioxidant, and anti-inflammatory properties. Initial screening indicated several extracts with notable α -glucosidase inhibitory activity. Total polyphenol content ranged from 116.966 ± 1.326 mg gallic-acid equivalents/g dry weight to 336.393 ± 10.222 mg/g extract, and the infusions displayed strong free radical-scavenging capacities in DPPH, nitric oxide, and superoxide anion assays. Among them, extracts from *Sanguisorba minor*, *Viburnum tinus*, and *Cistus salvifolius* exhibited especially promising bioactivities, although *Cistus salvifolius* was cytotoxic at higher concentrations. Overall, these findings highlight SENP's flora as a valuable source of natural compounds that merit further investigation for their potential in developing novel interventions for diabetes and oxidative stress.

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P154 Hepatoprotective potential of *Boerhavia diffusa* L. against alcoholic liver disease in HepG2 cells: An *in vitro* and *in silico* study

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Boerhavia diffusa Linn. (Nyctaginaceae) is a medicinal plant widely used in Ayurveda and traditional medicine across Africa and South America [1]. In India, its root decoction is commonly used to treat cirrhosis, jaundice, and hepatitis [2]. While previous studies have demonstrated its hepatoprotective potential *in vivo*, the

precise mechanisms remain unclear. A rotenoid-enriched fraction (REF) from *B. diffusa* roots was prepared as described previously by us [3]. In this study REF is being evaluated for hepatoprotective effect against ethanol-induced toxicity in HepG2 cells with silymarin as a reference standard and elucidated its molecular mechanism through an *in silico* approach. Hepatoprotective activity was assessed by measuring reactive oxygen species (ROS) levels (DCFH-DA assay), lipid accumulation (Oil Red O staining) and inflammation (nitric oxide assay). Alcohol metabolism was analysed through enzymatic assays for alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH). Molecular docking studies explored interactions of boeravinones with key hepatoprotective targets, including CYP2E1, NRF2, SIRT1, SREBP1, TNF- α , and TLR-4. REF (100 and 150 μ g/mL) exhibited significant hepatoprotective effects, reducing lipid accumulation ($23.22 \pm 1.09^{***}$ # and $12.24 \pm 0.98^{***}$ #) and ROS levels ($223.22 \pm 1.09^{***}$ # and $182.24 \pm 2.98^{***}$ #), comparable to silymarin. It also enhanced alcohol metabolism by increasing ADH ($66.21 \pm 1.14^{**}$ # and $82.67 \pm 0.95^{***}$ #) and ALDH activity ($65.88 \pm 1.56^{**}$ # and $80.22 \pm 1.67^{***}$ #). *In silico* analysis revealed strong interactions of boeravinones with hepatoprotective targets, with higher binding affinities than silymarin. REF exhibited potent hepatoprotective effects by mitigating oxidative stress, reducing lipid accumulation, and enhancing ethanol metabolism. The findings highlight its therapeutic potential for alcohol-induced liver disease and support further investigation into its clinical applications.

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P155 Assessing the potential fasting and postprandial mechanisms involved in the acute hypoglycemic effect of four plants used in traditional Persian medicine

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In traditional Persian medicine (TPM), herbal infusions are often used to treat hyperglycemia, called 'dam-kardeh'. Iranians traditionally use *Eryngium bungei* Boiss. (E. b), *Tragopogon buphthalmoides* (DC.) Boiss. (T. b), *Salvia hydrangea* DC. Ex Benth. (S. h), and *Juniperus polycarpus* K. Koch. (J. p) to control diabetes. However, their effectiveness in controlling glucose levels remains unproven. Our study aims to investigate the potential hypoglycemic effect of plant infusions during fasting and/or postprandial states as well as their mechanisms of action for future research. In the fasting state, T. b, S. h, and E. b were as effective as glibenclamide in lowering blood glucose levels in hyperglycemic rats. Moreover, all three suppressed G6Pase and FBPase enzymatic activity by 90–97 % and 80–91 %, respectively. However, they also demonstrated significant postprandial hypoglycemic efficacy. T. b caused a reduction comparable to the therapeutic efficacy of repaglinide (AUC). These extracts significantly inhibited PTP-1B *in vitro* (> 70 %) when investigating possible mechanisms of action. All plant extracts showed no signs of acute toxicity. In HPLC-EM-SQ-TOF analysis, several compounds contributed to biological activity, including phenolic acids and flavonoid glycosides. The present study supports the traditional use of T. b, E. b and S. h for the control of diabetes in the fasting and postprandial states. Additionally, these plants contain bioactive compounds with hypoglycemic and

antihyperglycemic properties. However, J. p only showed a modest effect in the fasting state and after 90 min. Chemical composition analysis and complementary experimental models are needed to expand these results.

P156 *In vitro* studies on the wound healing potential of dermatological plants from the Receptarium of Burkhard III von Hallwyl, a recipe text from 16th century Switzerland

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Considering the potential of medicinal plants to meet the need for additional effective measures in wound treatment, we pursued an ethnopharmacological approach to identify possibly wound-healing plants in the Receptarium of Burkhard III von Hallwyl (RBH), an extensive recipe text from 16th century Switzerland. Focusing on plants no longer used in modern practice, we mined RBH for specific historical uses apparently linked with the treatment of acute or chronic wounds. Seven corresponding plants were selected, from which aqueous and hydroethanolic extracts, as well as a press juice in one case, were prepared. All samples were investigated for their ability to stimulate migration of NIH-3T3 fibroblasts in a scratch assay (Agilent), while the LDH release was used as a measure to assess their cytotoxic profile.

Ten out of 17 samples showed an improved wound healing compared to untreated cells after 12 h of incubation. The most potent effects were observed for the aqueous extract of the leaves of *Sambucus nigra* L. at a concentration of 10 µg/mL with an improved wound healing of 59.14% ± 3.60 and for the press juice of the leaves of *Sempervivum tectorum* L. at 50 µg/mL of 39.44% ± 5.45. No cytotoxic effects were observed for these plants in the measured range (10 to 400 µg/mL) after an incubation time of 24 h. The results suggest an extended study of the promising samples and point to the value of historical texts as a source for natural product research.

P157 Phytochemical and pharmacological investigation of Ugandan medicinal plants traditionally used for wound healing

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Traditional medicinal plants are widely used in Uganda to treat wounds, typically as aqueous leaf-based formulations applied topically. However, scientific data on their phytochemical composition and therapeutic relevance remain limited.

This study aimed to identify key plant species traditionally used for wound healing in Uganda and evaluate their pharmacological and phytochemical potential for further development as improved traditional medicine. We compared wild-type and greenhouse-grown material as well as aqueous and alcoholic extraction methods in terms of extract composition and analytical quality. An ethnobotanical survey involving 70 traditional healers was conducted using semi-structured interviews and direct observation. *Bidens pilosa* L., *Hoslundia*

opposita Vahl, and *Plectranthus cyaneus* Gürke were most frequently cited, particularly for treating cuts and ulcers.

Aqueous and alcoholic leaf extracts (ethanol for *B. pilosa* L.; methanol for *H. opposita* Vahl and *P. cyaneus* Gürke) from wild and greenhouse material were analyzed. TLC, phytochemical screening, and HPLC-DAD revealed flavonoids, terpenoids, phenolic compounds, alkaloids, saponins, and anthraquinones. Alcoholic extracts consistently showed better separation, clearer metabolite detection, and richer HPLC profiles. Wild and greenhouse plants yielded similar results. A characteristic fingerprint region could be recorded for all extracts via HPLC-DAD, providing a reproducible basis for future extract quality control. Antimicrobial activity was observed in 10 of 48 extracts. Cytotoxicity assays (HaCaT keratinocytes, XTT/LDH) indicated no toxicity below 500 µg/mL. Ongoing analyses include LC-MS/MS, GC-MS, ELISA, and HaCaT migration assays to further characterize wound-relevant activity.

These findings support the development of improved traditional formulations through local cultivation, standardized extraction, and phytochemistry-guided approaches.

P158 Herbal Medicinal Products – Ethical considerations towards a better representation in medical guidelines

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Medical guidelines play an increasing role in therapy, and more and more herbal medicinal products are included (e.g. [1, 2]). Still, while e.g. the evidence of NSAIDs has been originally only characterized in pain after tooth extraction, they are now established for a broad range of pain syndromes, while herbal medicinal products (HMPs), despite having, based on their tradition of use and their pharmacology, an equally broad field of application, tend to be only considered in guidelines when a study in the specific pain syndrome is available. A similarly narrow approach can be observed for adjuvant therapies in oncological patients. For a recommendation of respective HMPs to additional syndromes, accordingly for each of them an own randomized placebo controlled clinical trial (RCT) would be needed, while ethical considerations tend to support a reduction of the number of RCTs to a minimum [3].

Concepts to extrapolate clinical evidence to adjacent patient groups where searched and assessed. A guideline recently issued by ICH, the guideline on the extrapolation of clinical data from adult to pediatric patients (E11A) [4, 5] could be a model. There, in the benefit-risk assessment of an extrapolation, three questions are raised: How similar is (1) the disease, (2) the pharmacology of the drug, and (3) the response to therapy, considering the similarity between the two patient groups as a continuum.

Transferring this systematic approach from the pediatric extrapolation also to the extrapolation of the evidence on HMPs between other patient groups could make these products with their often favorable benefit to risk ratio available for additional patient groups, without the need of additional, ethically sometimes questionable RCTs.

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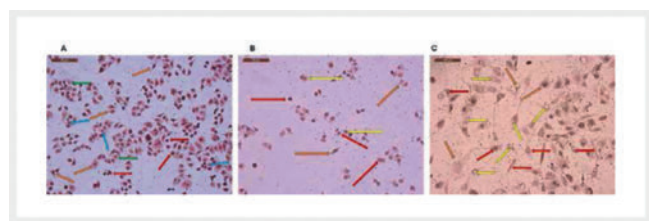
P159 Cytotoxic and antiproliferative effects of *Sechium edule* extract on cervical cancer cell lines: A natural approach to gynecologic oncology

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Cervical cancer remains the second leading cause of cancer-related death among women worldwide [1]. *Sechium edule* (chayote) has garnered attention due to its reported biological properties, including anti-inflammatory, antiproliferative, and anticancer effects [2]. We aimed to evaluate the cytotoxic activity of water-soluble phenolic compounds extracted from *Sechium edule* on HPV-positive (HeLa and CaSki) and HPV-negative (ViBo) cervical cancer cell lines. Chayote fruits were collected, dried, ground, and subjected to maceration for extract preparation. Phytochemical screening and thin-layer chromatography revealed the presence of secondary metabolites, particularly alkaloids and tannins. Cytotoxicity was assessed using MTT and Crystal Violet (CV) assays at concentrations of 0.75, 1.25, 2.5, and 5 µg/µL, and morphological changes were evaluated through hematoxylin-eosin staining. Results demonstrated a dose-dependent reduction in cell viability, with the most significant cytotoxic effect observed at 5 µg/µL, reducing viability to 24 % in CaSki, 51 % in HeLa, and 25 % in ViBo cells. Morphological analysis indicated characteristic features of cell death, ► **Fig. 1**. In conclusion, the *Sechium edule* extract exhibits potent cytotoxic activity against HPV-positive and HPV-negative cervical cancer cell lines, supporting its potential as a natural therapeutic agent in gynecologic oncology.



► **Fig. 1** Micrographs of cervical cancer cell lines (A. CaSki, B. HeLa, and C. ViBo) treated with 5 µg/µL of *Sechium edule* extract and stained with hematoxylin-eosin. Stained nuclei (blue), cytoplasm (green), membrane rupture (orange), membrane pores (yellow), and the presence of apoptotic bodies (red).

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P160 Neuroprotective effects of Gelsemium in tauopathy: Restoration of mitochondrial function and cellular bioenergetics in P301L human neuroblastoma cells

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Mitochondrial dysfunction is a pivotal factor in the pathogenesis of neurodegenerative diseases, such as Alzheimer's and Parkinson's. These disorders are often marked by energy deficits, oxidative stress, and compromised cellular maintenance mechanisms in the brain. Low-dose of *Gelsemium sempervirens* L. (Gelsemium) preparations at centesimal dilutions enhanced neurite outgrowth *in vitro*, likely by modulating mitochondrial activity and improving cellular energy balance [1]. Recently, we showed that Gelsemium preparations may have neuroprotective effects against stress-induced cellular changes in the brain by regulating mitochondrial functions, essential for the survival, plasticity, and function of neurons in depression [2]. The present study seeks to evaluate the ability of Gelsemium preparations to mitigate mitochondrial dysfunction on cell survival, ATP production, mitochondrial respiration and membrane potential in a human cellular model of Alzheimer's disease (AD). To achieve this, we studied the effects of selected Gelsemium preparations (3CH, 5CH and 30CH) using a sophisticated AD cell model, P301L cells, neuroblastoma cells stably transfected with human mutant tau (P301L mutation). Our results showed that 3CH, 5CH, 30CH exerted neuroprotective effects by i) improving viability, ii) regulating specific levels of mitochondrial or total superoxide anion radicals and total nitric oxide levels, iii) increasing ATP production, enhancing mitochondrial membrane potential, iv) shifting the bioenergetic profile towards a more metabolic state, in control and P301L cells, with the optimal treatment period being 24 hours. These findings provide promising insights into the neuroprotective properties of Gelsemium and its role in modulating mitochondrial function, oxidative stress and cellular metabolism in the context of tau-related neurodegenerative diseases.

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P161 *Salvia fruticosa* Mill. extract rich in carnosic acid in mild to very severe adult facial seborrheic dermatitis: a small case series

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Seborrheic dermatitis (SD) is a common inflammatory lifelong disease characterized by relapses and recessions that appears mostly on the face [1]. We report 10 patients (8 males, 2 females, age range: 40–78 years), suffering from mild to very severe facial SD (Seborrheic Dermatitis Area and Severity Index (SEDASI) average was 19, range = 10–60) who were treated with an O/W cream containing a leaf extract of *S. fruticosa* rich in carnosic acid (CA) [2], applying it to lesioned skin and around it, once at night. No concurrent therapies were used. There was a follow-up ranged from 0.5 to 4 years. To our knowledge, in the worldwide literature such a study has been reported only by our team. All patients experienced a complete clearance of their disease in short time (SEDASI = 0). The mean time to achieve this result was 7.7 days (range: 3–15 days). The follow up showed that clear state lasted for a long time. However, when

relapses occurred, patients responded well to retreatment in less time than initially needed. No adverse effects were observed during the treatment. Patients who followed maintenance treatment such as application of the cream once every night or day by day, did not relapse. We conclude that *S. fruticosa* leaf extract rich in CA is a potential natural agent for the topical treatment of SD as monotherapy, with high efficacy, able to induce disease clearance and long-lasting remission, without side effects. Moreover, it showed preservation of its efficiency and safety when treating relapses.

Conflicts of interest P. Kallimanis has a patent that covers the synthesis of the herbal product.

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P162 *In vitro* and *ex vivo* metabolism and intestinal permeability of dittany (*Origanum dictamnus* L.) infusion and its components

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Origanum dictamnus L. (Lamiaceae) is registered in EMA against cough, gastrointestinal disorders and skin inflammations and bruises [1]. In Crete, where the herb is endemic, it is used as a beverage for the stomach, as well as in dysmenorrhea. The purpose of this research was to assess how digestive processes affect the infusion, as such data lack. Four experiments were conducted: i) *ex vivo* intestinal permeability assay, ii) *in vitro* simulated digestion, including oral, gastric and intestinal phase, and iii-iv) stability test of the infusion (blank experiment) in the absence/presence of intestinal tissue. Ten constituents were considered for the study and were assessed by HPLC-PDA-MS. The intestinal permeability test showed that the Salvianolic acid P ring-open product, forming during the preparation of the infusion, had the highest permeability, reaching 12.59 µg/cm². *In vitro* digestion indicated that the constituents undergo enzymatic degradation during both gastric and intestinal phases, with the effect being more pronounced in the intestinal phase. In the aqueous media that simulated the intestinal environment (experimental conditions, i.e. buffer, temperature, oxygen, etc.) lignans showed the highest degradation (50–60%), while in the presence of intestinal tissue further breakdown is observed for all the compounds, suggesting that the bacterial enzymes play a major role in the decomposition of the components. This work represents the first study of the metabolism of the *O. dictamnus* infusion. The results indicated which compounds could be identified as potentially active principles and set the basis for further evaluation of the therapeutic potential of the herb.

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P163 Effects of the compound N-methyl iodide, isolated from *Brassica rapa* L. *in vitro* on HEK-293 human liver kidney and human breast and colon cancer cells

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Brassica rapa L. is an edible, cultivated vegetable and medical plant widely consumed since ancient times by different indigenous groups [1]. Recently, *Brassica rapa* L. was found to have anticancer, antimicrobe and anti-oxidation activities [2]. This research aimed to explore the antioxidant activity, anti-proliferative and anti-inflammatory properties of N-methyl iodide, a compound isolated from *Brassica rapa* L. on normal HEK-293, MCF-7 breast cancer, and HCT-116 human colon cancer cells stimulated with tumour necrosis factor alpha (TNF-α). N-methyl iodide compound was isolated from dichloromethane:methanol fraction extracted from dried leaves *Brassica rapa* L. Morphological assessment, cytotoxic activity and antioxidant capacity of the compound (10–200 µg/mL) were determined using light microscopy, tetrazolium-based colorimetric MTT and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays, respectively. The compound was not toxic to HEK-293 and the two cancer cells with LC50 > 200 µg/mL. Stimulation with TNF-α reduced the % viability of cancer cells significantly compared to non-stimulated cells. The compound aggravated the TNF-α-induced MCF-7 breast cancer cell death significantly with the 100 and 200 µg/mL. The compound did not alter the morphological structure of cells in comparison to doxorubicin (positive control). The compound had weak ABTS scavenging activity. The findings from this study suggested that the isolated compound N-methyl iodide, was not responsible for the anticancer and antioxidant effects presented with crude extracts of the plant. However, the study also showed that N-methyl iodide may have effects on the TNF-α anti-inflammatory pathways that could be advantageous in cancer treatment.

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P164 Gastroprotective and antiulcer effects of Korean melon

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Korean melon, botanically classified as *Cucumis melo* var. *makuwa*, commonly known as Chamoe in Korea. This study aimed to investigate the gastroprotective effects.

After juicing the melon, the remaining pulp was extracted with 65% ethanol. The resulting extract was concentrated under reduced pressure and lyophilized to yield Korean melon ethanol extract powder (ME). Sprague-Dawley rats were randomly divided into five groups of six animals each. Group A served as the normal control, while Group B received an oral dose of 1 mL of 150 mM HCl in 70% ethanol to induce gastric injury. Groups C and D were pre-treated with ME at doses of 60 mg/kg and 30 mg/kg body weight (p.o.), respectively, prior to HCl/ethanol administration. Group E was orally administered Stillen (60 mg/kg) as a positive control.

ME treatment significantly ameliorated gastric mucosal damage induced by 150 mM HCl/70 % ethanol, as confirmed by histological evaluation. The extent of gastric mucosal injury and the ulcer index were assessed through visual and histological examination of the inflamed gastric tissue.

ME treatment reduced the ulcer index in a dose-dependent manner by preventing mucosal lesions, erosion, and cellular degeneration. Additionally, ME-treated groups showed downregulation of the H^+/K^+ -ATPase pathway, as well as reduced expression of cholecystokinin-2 receptors (CCK2R), muscarinic receptors (M3R), and histamine receptors (H2R), all of which are associated with gastroprotective effects. These findings suggest that ME has the potential to be developed as a therapeutic agent for the treatment of acute gastritis.

P165 Indigenous medicinal plants used to treat shingles (*Herpes Zoster*) in Lepelle-Nkumpi, South Africa

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Indigenous medicinal plants are a solution to various diseases such as shingles, *Herpes Zoster* (HZ), particularly in rural areas with limited access to conventional healthcare [1]. The study aimed to explore the efficiency of indigenous medicinal plants used to treat HZ in Lepelle-Nkumpi, of Limpopo Province, South Africa. An ethnobotanical survey was conducted using semi-structured questionnaires from 35 traditional health practitioners selected purposively across five villages. Ethnobotanical indices such as Relative Frequency of Citation (RFC) and Fidelity Level (FL) were used for data analysis. A total of 11 plant species from 11 families were recorded for treating HZ. The highest RFC values recorded were *Ardenia spinosa* Burt Davy (0.257), *Aloe succotrina* Weston (0.228), and *Lepidonia callilepis* (Gleason) H. Rob. & Funk (0.229), indicating their widespread use. In terms of FL, *A. spinosa*, *Enicostoma axillare*, and *Gnidia caffra* Meisn had a high level of 100 % showing unified community preference. Among the recorded families, Passifloraceae stood out as the most prevalent [2]. The most utilized plant parts were roots [3] (46 %) and decoction (81 %) being the primary preparation method and the common mode of administration was oral (58 %). The majority of the plants were classified as least concern (73 %), followed by not threatened (18 %) and near threatened (9 %). The study highlights HZ's dependency on medicinal plants for health care. This suggests an awareness of medicinal plant conservation, use and promoting sustainable harvesting practices. Furthermore, more studies on biological assays and phytochemical compounds should be done.

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P166 Ethnobotanical importance of *Ziziphus mucronata* Willd. from Mphambo Village, Limpopo Province, South Africa

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Most of rural African people depend on indigenous medicinal plants for healthcare [1]. In South Africa many people are still struggling to find medical help due to distance and high cost, hence they opt for indigenous use of medicinal plants [2]. *Ziziphus mucronata* is one of the plants with potential medicinal uses in Southern Africa. The study aimed at investigating the indigenous medicinal uses of *Z. mucronata* from Mphambo village, Limpopo Province, South Africa. This study has hypothesised that people in Mphambo village have the indigenous knowledge of *Z. mucronata*. A survey was conducted with 50 participants by using purposive sampling and semi-structured interviews (faceto-face). Ethnobotanical indices such as Relative Frequency of citation and Use-value were used for data analysis. About 18 diseases and 9 different categories of diseases were recorded. Plants parts which are mostly used were leaves (35 %), followed by the roots (32 %), whole plant (15 %), bark (12 %) and fruits (6 %). Maceration (44 %) and decoction (33 %) were the most main preparation methods, and plant remedies were mainly administered orally (68 %). This study is in agreement with some previous studies where indigenous medicinal plants can be used to treat and manage some diseases. Therefore, this study might bring hope to people of Mphambo village to continue using and conserving *Z. mucronata*.

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P167 Nomilin counteracts glucose-induced mitochondrial dysfunction in *Caenorhabditis elegans* model of obesity

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Mitochondrial dysfunction plays a crucial role in the development of cardiovascular diseases, metabolic syndrome, obesity, diabetes, and neurodegenerative disorders. Natural triterpenoid compounds have been reported with diverse pharmacological properties, including anti-inflammatory, anti-obesogenic, and anti-aging effects [1]. Nomilin, a bitter-taste limonoid that is enriched in citrus peels, has been identified as a human pregnane X receptor (hPXR) agonist that extends lifespan and improves healthspan [2]. The data obtained in our preliminary studies has confirmed that nomillin reduces lipid accumulation in a nuclear factor erythroid 2-related factor 2 (NRF2)-dependent manner. Here, we aim to gain a more profound characterization of the effect of nomillin (100 μ M) on mitochondrial bioenergetics by utilizing a glucose-induced obesity model in *Caenorhabditis elegans* [3].

We assessed the changes in the lipid storage in *C. elegans* transgenic strains fed on elevated glucose through GFP-based confocal fluorescent detection. Furthermore, we evaluated the changes in mitochondrial dynamics through the MitoView Green and TMRE co-staining to enhance our understanding of the nomilin mode of action.

Our results revealed that nomilin enhanced mitochondrial dynamics, lipid beta-oxidation in the mitochondria and genes related to AMPK/SIRT signalling pathway in glucose-induced obesity model in *C. elegans*.

Taken together, we have provided insights on the molecular mechanism that drives the anti-obesogenic effect of nomilin and its role on mitochondrial bioenergetics regulation.

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P168 Investigation of antioxidant, antimicrobial, and anti-inflammatory activity of the Thai traditional extract “Benjalotika” and its five component herbs

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The Thai traditional herbal extract “Benjalotika” (Thai: @) (BK) contains five herbs: *Dracaena loureiroi* Gagnep. (DG), *Tarenna hoensis* Pit. (TP), *Mansonia gagei* J. R. Drumm. ex Prain (MP), *Tacca chancieri* Andre (TA), and *Cyathea podophylla* (Hook.) Copel (CC). Benjalotika extract is a traditional anti-inflammatory and antipyretic. This study investigates and compares the antioxidant, antimicrobial, and anti-inflammatory activity of the ethanolic extract of BK as well as the extracts of each component. All six extracts were prepared by maceration in 95 % ethanol, and each extract was dried by evaporator. All extracts were tested for antioxidant activity by DPPH assay and for antimicrobial activity by disc diffusion and broth dilution methods, both against *Staphylococcus aureus* (*S. aureus*), *Streptococcus bovis* (*S. bovis*), and *Escherichia coli* (*E. coli*). Anti-inflammatory activity was tested by nitric oxide (NO) inhibition (Griess assay, including MTT assay) on macrophage cells in murine (RAW 264.7). Results showed that among the six extracts, MP extract had the highest antioxidant activity (EC₅₀ value 4.82 ± 0.087 µg/mL) and also the highest antimicrobial activity, with an *S. aureus* inhibition zone of 17 mm and a MIC = 0.625 mg/mL against both *S. aureus* and *S. bovis*. MP extract also showed the highest anti-inflammatory activity during NO inhibition (IC₅₀ value 87.18 ± 4.12 µg/mL). MP extract (and all five other extracts) is non-toxic in RAW264.7 cells according to the MTT assay. In summary, these results validate and support the use of the traditional Benjalotika herbal extract for the treatment of infection and inflammation.

P169 Ethnobotanical insights into medicinal plants for managing childhood diseases and promoting wellbeing in South Africa

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In South Africa, approximately 3,000 plant species have been used for ethnomedicine throughout history [1]. Rural populations often prefer traditional

medicine due to its accessibility, affordability, and minimal side effects. Additionally, approximately 12 million children under five die annually in developing countries [2], with 70 % of these deaths caused by communicable diseases and malnutrition [3]. However, the potential of traditional medicine, particularly in managing childhood diseases, remains understudied [4]. This review assesses the use of plants for childhood disease management and well-being in South Africa. Data was collected from scientific databases, ethnobotanical books, and interviews. A total of 252 plant species from 72 families are documented as remedies across seven provinces, including *Aptosimum elongatum*, *Bulbine frutescens*, *Commelina diffusa*, *Euphorbia prostrata*, *Kigelia africana*, *Rhoicissus tridentata*, *Gomphocarpus fruticosus*, and *Vachellia karroo* [5]. These plants are recognized for their medicinal value in treating various childhood ailments in the region. The region does not specify thirty-two percent of the plant parts, with leaves (26 %) and roots (19 %) following closely behind. Topical application is the preferred method, especially for treating skin conditions, accounting for 75 % of usage. The findings highlight a rich wealth of indigenous knowledge regarding medicinal plants, suggesting that if properly explored, low-cost bio-products could be developed. These could improve livelihoods and help alleviate poverty in rural communities. However, further scientific research is needed to assess the efficacy and safety of these plants for pharmacological use.

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P170 Phytochemical profiling of different plant parts of *Trachyandra ciliata* (L.F) Kunth cultivated under varying degrees of salinity for bio-saline agriculture

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Wild edible halophytes are understudied in African countries, including South Africa. Thus, it is crucial to explore these species as potential therapeutic agents for the treatment of chronic diseases. *Trachyandra ciliata*, an underutilized edible halophyte, has shown potential as a nutritious vegetable under salinity conditions. However, its medicinal value is yet to be explored. Thus, the current study was conducted to profile bioactive compounds found in roots, leaves, and flower bud extracts of *T. ciliata* to explore their potential therapeutic uses. Plants were cultivated under four saline (NaCl) concentrations (50, 100, 150, and 200 mM), while the control was irrigated with the nutritional solution only. The Ultra-High Performance Liquid Chromatography Mass Spectroscopy (UHPLC-MS) was employed to quantify and characterize metabolites in leaves, roots, and flower buds of *T. ciliata*. The UHPLC-MS identified 71 compounds (including two novel compounds), which were grouped into flavonoids, anthocyanins, alkaloids, nucleobase, nucleosides/tide, saccharides, fatty acids, ami-

no acids, and coumarins. The diverse compounds identified show the extracts of *T. ciliata* could have biological activities against chronic diseases such as diabetes, neurodegenerative disorders, cancer, cardiovascular diseases, and gastrointestinal disorders. Results from this study further show the potential of this plant in the treatment of other ailments such as skin problems, viral diseases, inflammation, oxidative stress, as well as plant-based food additives and preservatives. These results support the use of *T. ciliata* as a therapeutic agent in the treatment of various ailments.

P171 *In vitro* antioxidant, cytotoxicity and anticancer potential of *Dichrostachys cinerea* (L.) Wight & Arn. dried leaf and stem extracts on MCF-7 human breast cancer cells

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Focus on traditional medicinal plants for their potential use in cancer therapy has increased. Imbalance between production of free radicals and reactive metabolites is a problem in cancer. This study evaluated the *in vitro* antioxidant, cytotoxic, morphological effects and anticancer properties of the leaf and stem extracts of *Dichrostachys cinerea* (L.) Wight & Arn., commonly known as Sickie bush on HEK-293 human embryonic kidney and MCF-7 human breast cancer cell to support their potential use in cancer treatment. Leaves and small-stems were dried and extracted with methanol, acetone, hot-water, and ethanol. Free-radical scavenging activity was assessed using 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay. Cytotoxicity and anticancer potential of the four extracts were evaluated using the tetrazolium-based colorimetric assay. Morphological analysis was done on the various concentrations of the 4 extracts and analyzed using light microscopy. Methanol, ethanol and water extracts had ABTS radical scavenging ability with IC₅₀ of 0.17 ± 0.08; 42 ± 6 and 67 ± 4 µg/mL, respectively. The results showed that all four extracts exhibited low cytotoxicity towards HEK-293 kidney cells, as their LC₅₀ values were greater than 150 µg/mL. Methanol extract changed morphological structure of the MCF-7 breast cancer cells with all the concentrations (20–200 µg/mL) similar to doxorubicin, positive control and toxic at 50 µg/mL, while the ethanol extract only changed morphology of the cancer cells from the 100 µg/mL concentrations. Acetone and hot-water extracts did not induce any morphological changes on the cell structures. Study demonstrated that methanol extracted compounds with potential high radical-scavenging ability and anticancer effects.

P172 Ethnopharmacological study of plants used against various diseases in the region of Northern Epirus, Albania

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In Balkan countries, people still resort to traditional medicine as herbal plants are more easily accessible and often more cost effective for the locals. In the case of Northern Epirus, its rich flora biodiversity along with the knowledge of traditional medicine passed orally through the generations could be the key to

the discovery of remarkable therapeutic agents. Under the European project "ETHICSTWIN", an ethnobotanical study was carried out among Albanian, Aromanian and Greek communities living in the areas of Northern Epirus, Albania, regarding the traditional uses of wild plants possessing nutritional and medicinal value and home-made medical remedies. More specifically, the survey was conducted in 16 villages by interviewing 28 locals, including individuals from each community. After processing the data of 84 questionnaires completed, 120 plant taxa were found to be used as nutrition agents as well as for treating numerous diseases such as *Allium cepa* L., *Cornus mas* L., *Crataegus monogyna* Jacq., *Hypericum perforatum* L., *Matricaria chamomilla* L., *Origanum vulgare* subsp. *hirtum* (Link) A. Terracc., *Punica granatum* L., *Rosa × damascena* Herm., *Salvia officinalis* L., *Sideritis raeseri* Boiss. & Heldr., *Urtica dioica* L., *Vitis vinifera* L. Through the comparison of scientific data found in literature with the information obtained from our study, it was demonstrated that the traditional use of the majority of the mentioned plants can be justified, highlighting the crucial role of Ethnobotanical research in providing the required data for both implementing community-based management of the local natural resources and further developing small-scale markets of local herbal and food products.

P173 Inhibition of *Staphylococcus aureus* and methicillin-resistant *S. aureus* by *Terminalia arjuna* fruit extract and its synergistic interaction with ampicillin

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Terminalia arjuna is in the Combretaceae family. Its fruit is a plant ingredient in Thai traditional formulas for treating phlegm and gastrointestinal tract diseases. A prior investigation demonstrated that *T. arjuna* bark extracts exhibit strong inhibitory effects against *Staphylococcus aureus* [1]. This research aimed to examine the antibacterial efficacy of *T. arjuna* fruit extract against *S. aureus* and methicillin-resistant *S. aureus* (MRSA). Furthermore, a study examines the interaction between *T. arjuna* extract and ampicillin in inhibiting *S. aureus* and MRSA. The findings indicated that the ethanolic extract of *T. arjuna* fruit inhibited *S. aureus* and MRSA, exhibiting a minimum inhibitory concentration (MIC) of 0.625 mg/mL. Ampicillin demonstrated an inhibitory action against *S. aureus* and MRSA, with MIC values of 0.078 and 200 µg/mL, respectively. Additionally, the co-incubation of *T. arjuna* fruit extract with ampicillin had a synergistic effect *in vitro* against MRSA, providing a FIC index of 0.375 [2]. Conversely, they exhibited indifference to inhibiting *S. aureus*, indicated by a FIC value exceeding 0.5. The plant extract examined in this study may contain components that inhibit the resistance mechanism of ampicillin. The chemical components of *T. arjuna* fruit extract were studied. It comprised two hydrolyzable tannins, namely chebulagic acid and gallic acid, with respective contents of 18.65 and 1.48 % w/w. Hydrolyzable tannin has been documented to block efflux pumps and demonstrate a synergistic impact with β-lactams against MRSA isolates [3]. *T. arjuna* may be used together with β-lactam antibiotics to combat antibiotic-resistant microorganisms.

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P174 Seasonal variation of antioxidant activity and bioactive compounds in *Acer tegmentosum* stems

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Acer tegmentosum, traditionally used in East Asian ethnomedicine to treat liver-related disorders, is a deciduous tree whose stems are gaining interest for their pharmacological potential. To provide scientific evidence supporting traditional uses and optimize harvesting practices, this study evaluated the seasonal variation in antioxidant activity and phytochemical contents of *A. tegmentosum* stems. Monthly samples from May to September were extracted using 70% ethanol and analyzed for DPPH radical scavenging activity and levels of salidroside, catechin, and epicatechin gallate using HPLC. Antioxidant activity increased progressively with harvest time, from 51.4% in May to 70.1% in September at 40 ppm. Similarly, total phenolic content increased from 457.1 to 482.6 µg GAE/g. Among the quantified compounds, salidroside—a phenylethanoid glycoside known for hepatoprotective effects—showed a significant increase from 56.02 to 61.48 mg/g. In contrast, catechin and epicatechin gallate levels remained relatively unchanged. These results provide a scientific rationale for traditional knowledge recommending late-summer harvesting to enhance medicinal efficacy. The study highlights how seasonal factors influence the pharmacological quality of plant materials and reinforces the importance of aligning modern analytical techniques with ethnopharmacological insights. This research contributes to the evidence-based validation of herbal medicines and supports the sustainable development of functional botanical products derived from *A. tegmentosum*.

P175 Anti-inflammatory, antioxidant activities, and cytotoxicity against oral cancer of a remedy and its components in the Mukaroke scripture

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Oral cancer is among the ten most common types of cancer in Thailand [1]. In Thai traditional medicine, the Mukaroke scripture offers a remedy that relieves disorders similar to oral cancer. It has four ingredients: *Citrus aurantifolia*, *Curcuma longa*, *Trachyspermum ammi* and *Aucklandia lappa*. Previous studies have demonstrated that herbal components in the recipe inhibit cancer cells. However, no reports have specifically mentioned the formula. Therefore, this study investigated the cytotoxicity of oral cancer cells using the Sulforhodamine B (SRB) assay, evaluated the anti-inflammatory effect with the nitric oxide inhibitory assay, measured the antioxidant activity by the DPPH (1,1-diphenyl-2-picrylhydrazyl) scavenging assay, and determined the total phenolic content of water, vinegar and 95% ethanol extract. The results showed that 95% ethanol extract of *Curcuma longa* had the strongest antioxidant activity, with a half maximal effective concentration (EC₅₀) value of 9.58 ± 0.01 µg/mL. Thus, the remedy ethanol extract exhibited potent antioxidant activity compared to water and vinegar, as indicated by an EC₅₀ value of 61.31 ± 7.03 µg/mL, had anti-inflammatory activity with a half-maximal inhibitory concentration (IC₅₀) value of 17.10 ± 1.83 µg/mL and demonstrated cytotoxic effects against oral cancer. The remedy ethanol extract had a selectivity index of 4.09 for oral cancer cells. These findings indicate that the remedy ethanol extract exhibited antioxidant, anti-inflammatory, and cytotoxic activity against cancer cells. Consequently, the remedy should continue to be used in Thai traditional medicine as suitable for treating inflammatory diseases and oral cancer.

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P176 Traditional uses and chemistry of the cosmopolitan genus *Stachys* (Lamiaceae)

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The genus *Stachys* L. (Lamiaceae) is widely distributed and extensively used in traditional medicine across Europe, Asia, and Africa. In South Africa, several species are used as herbal teas and to treat respiratory and urinary ailments, fever, wounds, and pain. This study presents a comparative review of the traditional uses and chemistry of ten South African *Stachys* species, based on literature surveys and original chemical analyses, and contextualises them within the global body of knowledge. A total of 83 volatile and 24 non-volatile compounds were identified using HS-SPME-GC-MS and UHPLC-MS. Key compounds include linalyl acetate, limonene, caryophyllene oxide, verbascoside, and harpagide, many of which are shared with globally used species. Most South African species are traditionally consumed as herbal teas, suggesting that flavour and aroma may play an underappreciated role in their continued use. These findings raise important questions: Are *Stachys* species mainly used for sensory appeal rather than pharmacological efficacy? Are their perceived medicinal benefits grounded in cultural tradition or supported by biological activity? Can any African *Stachys* species be developed into new crops or functional products for the herbal tea or phytomedicine markets? By comparing local and international species, this work highlights both the chemical uniqueness and shared uses across the genus, identifying promising candidates for future product development and pharmacological study.

P177 Rediscovery of forgotten remedies: Exploration of ancient European texts for the identification of medicinal plants targeting metabolic diseases

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This study explores the rediscovery of medicinal plants and their traditional uses through the analysis of European manuscripts from the modern (c.1450–1800) and contemporary (post-1800) periods. These historical texts serve as valuable sources of information on natural treatments used for various diseases, notably metabolic disorders. Five books were selected and examined based on a compilation of medical terminologies related to liver diseases, diabetes, and obesity. The plant names identified in those texts were systematically cross-referenced with current botanical nomenclature using the International Plant Names Index (IPNI) to ensure accurate classification. Among the numerous plant species recorded, four—*Agrimonia eupatoria*, *Chelidonium majus*, *Geum urbanum* and *Alchemilla vulgaris*—were consistently cited across multiple manuscripts. Although historically recognized for their medicinal properties, these plants remain largely understudied in modern scientific literature, particularly in relation to their biological activities in the context of metabolic diseases. The next phase of this project will focus on evaluating the therapeutic and/or preventive potential of their extracts against metabolic diseases, with a particular emphasis on non-alcoholic fatty liver disease (NAFLD). This will be achieved by testing the extracts *in vitro* on hepatic cell cultures under lipotoxic conditions.

By integrating ancient knowledge with contemporary scientific methodologies, this research aims to identify new, ethical, and sustainable therapeutic solutions that highlight the value of local medicinal plants.

P178 The secrets of Witch Broom: A journey into traditional medicine and phytochemistry

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Fungal infections in trees often go unnoticed until visible signs appear. Certain *Taphrina* species can trigger metabolic changes in *Betula* (birch) genes, leading to tumor-like growths known as Witches' Broom (WB) disease. These distinctive deformities were traditionally used in Sami medicine to treat skin conditions [1]. Preliminary evidence suggests potential anticancer, anti-migratory, and anti-inflammatory properties of these extracts [2], though further scientific validation is needed. This study aimed to determine the chemical composition of Witch Broom (WB) and evaluate the validity of its traditional use in the treatment of skin diseases. Three samples were collected from Liverpool (UK), Berlin (Germany), and Helsinki (Finland). The aqueous extracts were analyzed using HPLC-DADMS, resulting in the identification of 30 compounds. The biological effects of the extracts on human keratinocyte (HaCaT) and melanoma (HTB-140) cells were assessed using an MTT assay. The results indicated lower cytotoxicity toward melanoma cells (IC_{50} = 487.5 μ g/mL) compared to keratinocytes (IC_{50} = 260.9 μ g/mL). A scratch assay demonstrated that a non-toxic concentration (62.5 μ g/mL) inhibited keratinocyte migration by ~95% after 20 h. Furthermore, ELISA analysis revealed a significant reduction in IL-6 levels following treatment with the WB extracts, while IL-8 levels remained unchanged. Finally, the antibacterial activity of WB extracts against common human skin pathogens was also evaluated. Our findings suggest that the traditional use of WB aqueous extracts in skin conditions may be scientifically justified. The extracts capacity to inhibit cell migration and exert selective anti-inflammatory effects points to its potential for development into novel therapeutic strategies for skin diseases.

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P179 Medicinal uses of local flora in the municipalities of Dombe Grande and Balombo, Benguela Province, Angola

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Africa is rich in natural resources and possesses a deep understanding of the uses and properties of plants, particularly in rural areas [1]. Traditional Medicine (TM) of African people is based on the heritage of knowledge about ethnop-

armacology. However, many medicinal uses of plants are yet to be validated, which opens many prospects for the discovery of new active principles, novel drugs or nutraceutical compounds [2]. Regarding Traditional Medicine Practitioners (TMP) and TM, Angola remains largely unstudied, thus offering a unique opportunity to discover previously unreported medicinal plants (MP) [3]. The main objective of this study was to document the medicinal uses of local flora in the municipalities of Dombe Grande (DG) and Balombo in the Benguela province of Angola. Given their very different edaphoclimatic characteristics and ethnolinguistic populations, we expected to find differences between them, enhancing the possibility to catalogue a greater variety of plant species [4]. An ethnobotanical survey was conducted in the two municipalities in 2024, in which TM practitioners were asked to answer a semi-structured questionnaire about the TM practices, diseases treated and plants used. A total of 124 species reported by the local TMP's were identified, 100 in Balombo and 24 in DG. The most widely used families were Fabaceae for Balombo and Acanthaceae for DG. The most common preparation methods were infusions and the main administration mode was ingestion. After applying the ICPC code classification to the reported diseases, it was found that the most common ailments fell under categories A (general and unspecific) and D (digestive).

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P180 Assessing the acute hypoglycemic effects of *Jefeá lantanifolia* (S. Schauer) Strother and its main compounds in rats

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Jefeá lantanifolia is traditionally used in the Mexican state of Hidalgo for T2D treatment. The aerial parts of the plant are used to prepare an infusion, which is drunk throughout the day. However, the effectiveness and active ingredients of this plant in controlling blood glucose levels are still unknown. This study aims to investigate whether traditional doses of infusion (AE) and ethanol: water (EWE) extracts and the main chemical constituents of *J. lantanifolia* have hypoglycemic effect in fasting and postprandial states in a STZ-NA-induced hyperglycemic rat model. Phytochemical studies led to the isolation and identification of luteolin (1); 2 β -hydroxy-dimerostemma brasiliolide-1-O-(3-hydroxymethacrylate) (2); homoplantaginine (3); cynarin (4), luteolin 7-O-glucoside (5) and nepitrin (6) as the main compounds. The extract was safe at 2 g/kg b. w. in acute toxicity studies. According to *in vivo* studies, all treatments markedly improved blood glucose levels in fasting states. Compounds 2 and 3 reduced blood glucose levels the most, by 42 and 40 %, respectively, while glibenclamide decreased blood glucose by 51 %. In the postprandial state, all treatments effectively reduced elevated glucose levels, particularly compounds 3 and EWE. Our findings provide preliminary support for the traditional use of *J. lantanifolia* as a complementary diabetes treatment in the fasting and postprandial states and highlight the main constituents as promising candidates for more research. Further studies are needed to conduct more thorough bio-

logical investigations, including long-term therapeutic efficacy as well as the use of higher concentrations of the compounds and the evaluation of potential risks associated with higher doses.

P181 Willow-deived antimicrobial against food-borne pathogenic bacteria

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Bacillus spp. are identified as the major causes of foodborne illness and diarrhea worldwide [1]. Willow species including *Salix starkeana* belong to the family Salicaceae, and were reported in European traditional medicine for their uses against gastrointestinal disorder, as well as for food packing application and preservation. Antimicrobial and phytochemical evaluations reveal the presence of promising antimicrobial constituents in willows extracts [2, 3]. This study aims to explore the anti-*Bacillus* activity of *S. starkeana*-derived components, their possible synergistic action with conventional antibiotics and their chemical constituents. Methanol extracts of *S. starkeana* twigs demonstrated MIC value at 625 µg/ml against the growth of *B. cereus*. Furthermore, when *S. starkeana* methanol extracts (9.7 µg/ml) combined with tetracycline hydrochloride (0.061 µg/ml), the results showed a synergistic effect with the FIC value of 0.13. This result paves the way for possible development of alternative and complementary antimicrobial therapies using sustainable plant-based extracts. The chemical composition of *S. starkeana* extracts, as analyzed by UPLC/QTOF-MS (negative ionization mode) revealed the presence of galloyl-glucose (331,0660), benzylprimeveroside (401,1453), salicortin (423,1293), acetyl salicortin (465,1397), procyanidin B1 (577,1357), and catechin (289,0704). Notably, two unknown compounds were identified for the first time in *Salix* species with the molecular ion at [M-H]⁻ 453,1753 and [M-H]⁻ 495,1511. This study demonstrates that *S. starkeana* could be used as an excellent source of natural food preservatives. Also, this result paves the way for the possible development of alternative and complementary antimicrobial therapies using sustainable plant-based extracts.

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P182 Hormetic response in *C. elegans*: effects on healthspan and physiological parameters

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The controlled application of low-dose stress, known as hormetic stress, has been shown to improve metabolic function and extend lifespan [1]. However, the precise “dose” of stress is critical for triggering adaptive responses and increasing biological plasticity [2, 3]. Moreover, certain natural compounds can trigger hormetic responses by activating adaptive stress pathways [4].

This study aims to investigate the effects of hormetic stress on metabolism and key pathways associated with obesity and aging in *Caenorhabditis elegans*, providing insights into its potential role in healthspan. By determining the specific dose ranges that trigger beneficial responses, we also seek to identify natural products capable of eliciting similar effects.

Our study reveals a complex pattern of endurance in worms exposed to mild (28 °C) and acute (30 and 35 °C) heat stress for varying durations (2, 4, and 6 h). Additionally, a hormetic cold stress response was assessed (4 °C for 1, 2, and 5 h), along with an oxidative stress response induced by paraquat (5, 10, and 25 mM) for 24 h. A dynamic shift in the worms’ survival and physiology was observed, reflecting a transition from detrimental to longevity-promoting effects under the applied stress conditions, thereby indicating possible hormetic responses.

These results highlight the potential of hormetic stress to regulate metabolism, emphasizing its significance in healthspan.

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P183 EGb 761® attenuates LPS-mediated inflammatory responses of microglial cells protecting neuronal cells *in vitro*

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Inflammatory processes in the brain mediated by infections, injuries or toxins contribute to cognitive decline. Neuroinflammation is typically triggered by the activation of immune-related cells like microglia which secrete pro-inflammatory cytokines, reactive oxygen species (ROS) or other neurotoxic substances. EGb 761®, a dry extract from *Ginkgo biloba* leaves, is used for the treatment of declining cognitive performance. The extract is adjusted to contain 22.0–27.0% ginkgo flavonoids, 5.4–6.6% terpene lactones and contains less than 5 ppm ginkgolic acids. To evaluate a possible anti-inflammatory effect of EGb 761®, we activated microglial cells (BV2 cell line) using lipopolysaccharide (LPS) and treated the cells simultaneously with different concentrations of EGb 761®.

LPS-induced the expression of pro-inflammatory mediators and enzymes such as *IL1b*, *IL6*, *Cxcl1*, *Cox2* and *Nos2*. Except for *IL6*, EGb 761® diminished or normalized the expression of the analyzed inflammation-related genes. This anti-inflammatory effect was confirmed on protein level. LPS-induced ROS burst was attenuated by EGb 761®. Interestingly, conditioned media from LPS-activated microglia induced neurotoxic effects in neuronal cells (Neuro2a) which was prevented by treatment of the neurons with EGb 761®.

Taken together, EGb 761® exhibits anti-inflammatory effects in a murine microglial cell line and protects from neural damage mediated by activated microglial cells *in vitro*. This data suggests that attenuation of neuroinflammation could contribute to the clinically observed beneficial effects of EGb 761® on cognitive decline in patients.

P184 Protective effect of tea silkworm feculae extracts on LPS/D-GalN-induced fulminant hepatitis

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Fulminant hepatic failure (FHF) is an acute onset of liver injury and hepatocellular dysfunction with poor prognosis and high mortality. Tea silkworm (*Andraca theae*, AT) larva feculae are utilized as a source of functional beverage soup or folk medicine for various indications. The objective of this study was to explore the bioactivity of AT larva feculae extracts against FHF in mice and the underlying mechanisms. The total ethanolic extract of larva feculae (FetOH) and its further enriched bioactive fraction 12 (F12) showed *in vitro* anti-inflammatory and antioxidative activities via downregulating NF- κ B pathway and proinflammatory cytokines and upregulating Nrf2 signaling pathway and HO-1 expression. FetOH and F12 treatment drove macrophages to M2-like phenotype in the LPS/IFN- γ co-stimulated RAW264.7 cells. The *in vivo* therapeutic effects of FetOH (100 mg/kg), F12 (20 mg/kg), and a reference drug silymarin (50 mg/kg) were investigated in LPS and D-galactosamine (LPS/D-GalN)-induced FHF mouse model. Both larva feculae extracts reduced hepatocytes apoptosis in liver tissues and levels of liver index enzymes aspartate aminotransferase and alanine aminotransferase and proinflammatory TNF α and IL-6 in sera of FHF mice which was compatible or superior to silymarin effect. Compared to the control mice, higher M2-like macrophage populations and neutrophils infiltration were observed in the FetOH- and F12-treated FHF mouse liver microenvironment. Notably, FetOH and F12 extracts also enhanced the phagocytic ability of primary neutrophils isolated from the tested mouse livers. This study provides evidence-based bioefficacy and molecular mechanisms of AT larvae feculae extracts on hepatoprotection revealing the great potential for FHF therapy.

P185 UPLC-qToF-MS metabolite profiling and cytoprotective activity of *Harpagophytum procumbens* hairy root extract in psoriasis model in human keratinocytes

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Psoriasis is a chronic inflammatory skin disorder with a profound impact on patients' quality of life. Current therapies are often accompanied by adverse effects and limited long-term efficacy. This necessitates the exploration of innovative, safer therapeutic options [1]. Natural products, particularly those enriched with secondary metabolites, represent a promising avenue in addressing such unmet medical needs [1, 2].

The study aimed to ultra-performance liquid chromatography – quadrupole time-of-flight mass spectrometry (UPLC-qToF-MS) metabolite profiling of a biotechnologically produced *Harpagophytum procumbens* (Devil's claw) hairy root extract (HPE) to confirm the presence of its main active secondary metabolites – verbascoside (VER) and leucosceptoside A (LEU A). Additionally, the cytoprotective properties of the extract in the context of psoriasis were assessed by monitoring reactive oxygen species (ROS) production in control and interferon (IFN)- γ /interleukin (IL)-17A/IL-22-stimulated human keratinocytes (HaCaT cells), serving as a model of psoriasis-like inflammation.

Both secondary metabolites, VER and LEU A, were tentatively identified in the HPE. Intracellular ROS accumulation was detected using a 2',7'-dichlorofluorescein-diacetate (DCF-DA) fluorescent probe [3] in control and psoriasis-modelled group treated with HPE, VER or LEU A. The results confirm that ROS pro-

duction is increased in IFN- γ /IL-17A/IL-22-stimulated keratinocytes and is partially modulated upon treatment. Therefore, the application of either HPE or its active constituents may help counteract excessive ROS production within the inflammatory environment in psoriasis pathophysiology.

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P186 Acute and sustained cognitive enhancement: The impact of *Sideritis scardica* and *Ginkgo biloba* – extracts and combinations thereof

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Cognitive enhancement is increasingly important as the population ages. Extracts from *Sideritis scardica* (Greek mountain tea) and *Ginkgo biloba* have shown potential cognitive benefits. This article explores the acute and sustained effects of these extracts on cognitive function. *S. scardica* originates from the eastern Mediterranean and is rich in polyphenols with antioxidant and anti-inflammatory properties [1]. Recent studies have investigated its impact on cognitive function. The acute effects of *S. scardica* extract appear to enhance attention, memory, and overall cognitive performance [2]. *G. biloba*, derived from the ancient ginkgo tree and used in traditional Chinese medicine, contains flavonoids and terpenoids. It improves blood flow, protects against oxidative stress, and enhances neurotransmitter function. Clinical trials show improved memory and attention acutely, and long-term use is linked to reduced cognitive decline [3]. Our study suggests that the combination may enhance cognitive performance beyond what each extract can achieve individually. The mechanisms underlying this synergy involve improved neurotransmission in the hippocampal area. Discriminant analysis of electro-encephalograms (EEG) data after *G. biloba* extract and *S. scardica* extract revealed a close neighbourhood among each other pointing to a very similar type of pharmacological action [4]. These data corroborate those obtained in individual controlled trials and long-term studies, which have found an increase in cerebral blood flow and reduction on neuroinflammation [5]. The mixture of the two extracts combining a polar-hydrophilic *S. scardica* extract and a lipophilic enriched *G. biloba* extract may be useful in treating neurodegenerative diseases and post-COVID-19 exhaustion, especially in ageing populations.

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P187 The impact of climate change on medicinal plants: A scoping review

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Medicinal plants are essential for human and animal healthcare, for ecosystems, and play important socio-economic roles [1]. However, climate change has threatened their supply and sustainability [2, 3]. This review maps the current state of research on how climate change affects medicinal plants, focusing on ecological shifts, traditional uses, changes in secondary metabolites, and adaptation strategies. 216 studies were analyzed, showing an increase in publication after 2021. Most research was conducted in Asia, especially China and India, while Europe, Africa and South America remain underrepresented. The review covers 373 medicinal plant species, including high-altitude, climate-sensitive species such as *Nardostachys jatamansi* D. Don DC. [Caprifoliaceae] and *Paris polyphylla* Sm. [Melanthiaceae]. Of these, 38.1% are classified as threatened by IUCN, and 61.9% remain unevaluated, pointing to significant conservation gaps.

Research methods varied from basic experiments to advanced computational approaches, notably species distribution modeling (SDM), particularly MaxEnt, the most commonly used. Although climate change is projected to increase habitat suitability for 70 species, it has reduced suitable habitats for 106 species, caused range shifts in 94 species, and placed 33 species at risk of extinction and habitat loss. Reduced availability has led to less effective substitutes in traditional use.

Heat, CO₂, low rainfall, and drought can lead to changes in secondary metabolites, including increased and decreased bioactive or marker compounds.

Climate change is reshaping medicinal plants' ecology and pharmacological value. While adaptation strategies exist, their implementation remains limited. An interdisciplinary, coordinated response is urgently needed to ensure sustainability and preserve traditional knowledge.

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P189 The variation of 6-gingerol content in ginger from different growth areas in Thailand

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Ginger (*Zingiber officinale* Roscoe, Zingiberaceae) is a medicinal plant rich in essential vitamins and minerals that benefit human health. It has long been utilized as a culinary spice and in traditional medicine to enhance the immune system and alleviate various ailments, including colds, asthma, allergies, fevers, and pain. Its active compound, gingerol, is important in its medicinal properties. This study aimed to investigate the 6-gingerol of ginger from different growth areas in Thailand. The ethanolic extract of 12 ginger was analyzed by measuring the 6-gingerol content using High-Performance Liquid Chromatography (HPLC). The results showed that the 6-gingerol content varied, ranging from 21.33 to 119.03 mg/g. Eight ginger, including GS01, GS02, GS03, GS04, GS05, GS06, GS08, and GS10, produced high levels of 6-gingerol, ranging from 82.38 to 119.03 mg/g. Specifically, CRI06, MSN04, and SSK01 produced more than 100 mg/g of 6-gingerol (100.90, 106.73, and 101.21 mg/g, respectively). These samples were found in soils with optimal characteristics for promoting 6-gingerol production, such as suitable levels of organic matter (ranged between 3.44 and 6.11 %), pH (ranged between 5.9 and 6.5), phosphorus (ranged between 144.0 and 384.2 mg/kg), potassium (ranged between 158.0 and 352.5 mg/kg), and nitrogen (ranged between 0.17 and 0.30 %). For the remaining four ginger, including GS07, GS09, GS11, and GS12, the 6-gingerol content was below 80 mg/g, which might be due to the incomplete quality of the cultivation area. This research suggests that soil quality is a key factor in the production of the compound [1].

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P190 Investigating the cardioprotective potential of *Punica granatum* L. juice in a *Caenorhabditis elegans* model of atherosclerosis

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Atherosclerotic cardiovascular disease is a leading age-related disorder and a major cause of morbidity and mortality worldwide. Its progression is driven by complex metabolic and inflammatory processes, yet effective interventions remain limited [1]. Emerging evidence suggests that dietary polyphenols play a crucial role in modulating key pathways associated with atherosclerosis [2]. Pomegranate juice (PGJ) is a rich source of polyphenolic compounds and has demonstrated various health benefits, including anti-inflammatory, neuroprotective, and stress-reducing effects. Additionally, it is considered a positive modulator of cardiovascular disease risk markers; however, its underlying mechanisms remain largely unexplored [3, 4].

This study aimed to develop a preclinical model of atherosclerosis-related metabolic dysfunction to investigate the potential of PGJ and its major constituent, punicalagin, in modulating metabolic healthspan and key energy-sensing pathways involved in disease progression. *Caenorhabditis elegans* serves as a powerful platform for studying lipid metabolism, oxidative stress responses, and longevity, making it an ideal system for cardiovascular research [4, 5].

Our findings reveal that PGJ significantly enhances metabolic healthspan by improving energy expenditure, reducing lipid accumulation, and increasing stress resilience to acute oxidative and thermal challenges. Additionally, pomegranate juice regulates key morphological parameters of *C. elegans*, suggesting broader effects on organismal physiology. Ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry analysis (UPLC-MS-qToF) identified punicalagin as the principal bioactive component, supporting its potential role in mediating these effects.

These findings provide novel insights into its therapeutic potential of PGJ for cardiovascular health.

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P191 Research and development of medicinal plant products in South Africa: Historical perspectives and current trends

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South Africa is a hotspot of botanical and cultural diversity and home to the most ancient lineage of modern humans, the /Xam people (Southern Bushmen). *In situ* selection (especially in the Cape region), of medicinal plants over potentially thousands of years have resulted in a unique medicinal culture [1] with several endemic plant species as the *materia medica*.

Well-known examples of South African plant species that have been commercialized and are now cultivated include *Agathosma betulina* (P.J.Bergius) Pillans (Rutaceae), *Aloe ferox* Mill. (Asphodelaceae), *Artemisia afra* Jacq. ex Willd. (Asteraceae), *Aspalathus linearis* (Burm.f.) R.Dahlgren (Fabaceae), *Bulbine frutescens* (L.) Willd. (Asphodelaceae), *Cyclopia genistoides* (L.) R.Br. (Fabaceae), *Harpagophytum procumbens* (Burch.) DC. ex Meisn. (Pedaliaceae), *Hoodia gordonii* (Mason) Sweet ex Decne. (Apocynaceae), *Lessertia frutescens* (L.) Goldblatt & J.C.Manning (Fabaceae), *Mesembryanthemum tortuosum* L. (Aizoaceae) and *Pelargonium sidoides* DC. (Geraniaceae) [2]. Ethnobotanical studies over the last decades have revealed several widely used but hitherto scientifically unexplored species with commercial potential as medicinal products and functional foods, such as *Dicerothermus rhinocerotis* (L.f.) Koek. (Asteraceae) – a traditional deodorant and flavourant, *Dicoma capensis* Less. (Asteraceae) – a traditional tonic and diuretic, *Diosma hirsuta* L. (Rutaceae) – once the most important original Khoisan buchu of the Cape, as yet poorly studied) and *Rafnia amplexicaulis* (L.) Thunb. (Fabaceae) – a traditional phytoestrogen.

The historical and contemporary processes through which South African plant species have become commercialized vary greatly [3]. Research and development activities are not only ongoing but also gaining new momentum [4].

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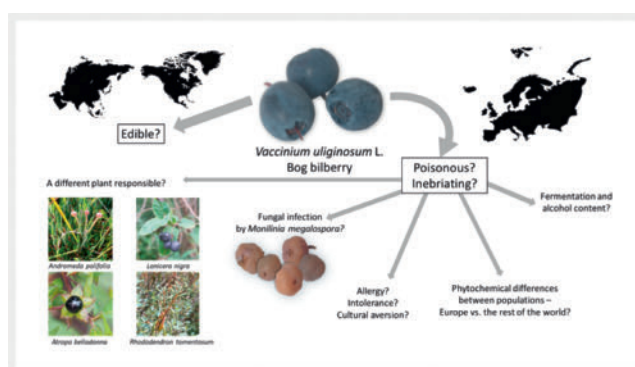
P192 The bog bilberry enigma: How phytochemistry unraveled a century-old mystery of *Vaccinium uliginosum* toxicity

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Closely related to other bilberries and blueberries, *Vaccinium uliginosum* (bog bilberry) is widely consumed in North America and Asia but has been historically avoided in the main parts of Europe. In the previously published historical literature review [1], several hypotheses for this phenomenon were postulated (► Fig. 1).



► Fig. 1 Hypotheses for the alleged toxicity of bog bilberries (adapted from Vaneková et al. 2024 [1])

The *Bog Bilberry Enigma* project aimed to address this discrepancy in a systematic way using a combined phytochemical and ethnopharmacological approach (UHPLC and UHPSFC coupled with PDA/MS, bioactivity testing on *Caenorhabditis elegans* and human liver cells GFP-HuH 7).

The findings revealed minimal phytochemical differences between European and North American samples, with no evidence of toxic alkaloids or harmful secondary metabolites. Extracts exhibited no toxic effects in *in vitro* cell viability assay on GFP-HuH 7 cells, nor in *in vivo* *C. elegans* survival assay. Berries infected by *Monilinia megalospora* showed altered flavonoid and anthocyanin content but no increased toxicity. Notably, bog bilberries demonstrated superior fermentation potential resulting in a significant alcohol content, thus potentially explaining historical accounts of inebriation. In conclusion, while direct toxicity derived from these fruits appears unlikely, factors such as human error, individual intolerance, or endophytic activity still need to be considered as possible reasons for the European aversion to bog bilberries.

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P193 Proanthocyanidins from *Ginkgo biloba* extract EGb 761® improve bioenergetics and stimulate neurite outgrowth *in vitro*

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EGb 761® is a proprietary extract from *Ginkgo biloba* leaves approved as a medication for the treatment of dementia and related disorders [1]. Preclinical studies highlight antioxidant, ROS scavenging, mitochondria-stabilizing and neuroplastic properties as some of the reported pharmacological activities [2]. Quantified active compounds in EGb 761® cover around 30 % of the mass balance and there is the possibility that additional compounds from residual 70 % may enhance the activity of EGb 761®. Proanthocyanidins (PACs) are a quantitatively relevant component in EGb 761® and some pharmacological activity has been reported for PACs from *Ginkgo* and other herbal sources [2–4]. Nevertheless, more insights are needed on precise mechanisms of the effects of PACs on a cellular level.

In this study, we focused on the effects of EGb 761® and its isolated PACs on mitochondrial bioenergetics and neuroplasticity in the human neuroblastoma cell-line SH-SY5Y. We successfully demonstrated the favorable effects of EGb 761® and its PACs fraction on mitochondrial bioenergetics, ROS scavenging and neurite outgrowth. Both our findings and prior research underline the ability of EGb 761® and PACs to modulate mitochondrial function, boost bioenergetics, decrease oxidative stress and enhance neurite outgrowth, thereby supporting neuroplasticity [2–5]. PACs and the main constituents of EGb 761®—flavonols, ginkgolides, and bilobalide—exhibit similar neuroprotective properties and complementary mechanisms of action, suggesting potential synergistic interactions among them. As PACs demonstrate pharmacological effects comparable to those of other key constituents, proanthocyanidins should be recognized as an essential bioactive component of EGb 761®.

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P194 Silexan® reduces neuroinflammatory-associated responses in LPS-stimulated microglial cells *in vitro*

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Neuroinflammation is driven by factors such as infections, brain injuries, toxic metabolites, and autoimmune responses. It can contribute to neurodegenerative diseases but also are associated with psychiatric diseases such as anxiety disorders and major depression [1, 2]. Silexan® is a proprietary essential oil from *Lavandula angustifolia* that has shown efficacy in patients with anxiety disorders and major depression [3, 4]. *In vitro* studies suggest neuroprotective effects on neuronal cells but its potential effect on neuroinflammatory processes has not been evaluated yet.

In this study we aimed to assess the effect of Silexan® on microglia activation parameters considered relevant for neuroinflammation.

Human (HMC3) and murine (BV-2) microglial cell lines were treated with different concentrations of Silexan® concomitantly with addition of LPS as an inflammatory challenge. At 24 h, chemokines, cytokines and production of reactive oxygen species (ROS) and nitric oxide (NO) were analyzed.

LPS induced inflammatory responses in microglia evidenced by an increase in interleukin-1 beta (IL-1β), Interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α) and chemokines. Treatment with Silexan® significantly reduced the expression and secretion of IL-1β, IL-6, IL-8 and CCL2 in human HMC3 cells and of IL-1β, IL-6 and TNF-α in murine BV-2 cells.

These results indicate an anti-inflammatory effect of Silexan® on the activation of microglial cells.

Our data suggest that attenuation of neuroinflammatory processes could potentially contribute to the therapeutic effects of Silexan® observed in patients with anxiety disorders and major depression.

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P195 No interaction between *Eleutherococcus senticosus* fruits intract and methotrexate in mice CB-17 SCID with induced human leukemia

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Eleutherococcus senticosus (Rupr. et Maxim.) Maxim. fruits and roots are used to treat immune-related diseases. Because of the overexploitation of the roots, the species is considered to be endangered therefore, the aerial parts of *E. senticosus* might be explored as a new sustainable source of compounds with an immunostimulative activity [1]. This study was aimed to evaluate the impact of the *E. senticosus* fruits intract on an effectiveness of chemotherapy in mice CB-17 SCID with induced human leukemia, in co-administration intract (*p.o.*) – methotrexate (*i.v.*).

Mice SCID (CB17/Jcr-Prkdc) were injected subcutaneously with MV4-11 leukemia cell line, then mice have been treated for 14 days with water (group I), methotrexate in dose 20 mg/kg b.w., *i.v.* (group II), intract in dose 750 mg/kg b.w., *p.o.* (group III) and intract + methotrexate, 750 mg/kg b.w., *p.o.* and 20 mg/kg b.w., *i.v.* (group IV). Tumors were measured 3 times a week. Euthanasia on the 15th day, next the standard blood and biochemical parameters were measured.

The intract did not have an impact on the chemotherapy effectiveness, as tumor's growth was slowed in both group II and IV. It means that the intract did not alter methotrexate activity. There were significant differences in ALT and AST activity between groups I and II versus III and IV, with a slight increase noted in groups III and IV.

Our observations emphasize the justification of the traditional use of the fruits in cancer-related diseases. At the administered dose, co-administration with

methotrexate appears to be relatively safe. Based on the obtained results, a dose of 750 mg/kg b.w. is recommended for further research.

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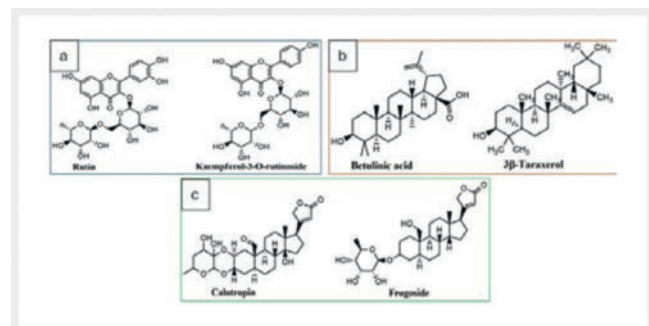
P196 Pharmacological uses and phytochemical properties of *Gomphocarpus* species: A bibliometric search from 1964 to 2024

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Gomphocarpus genus (Apocynaceae: Asclepiadoideae) is widespread across Africa, with 20 accepted species native to South Africa and other African countries [1]. Among the species, *Gomphocarpus fruticosus* (L.) Aiton is the most popular and has well-documented ethnopharmacology potential as a traditional medicine for various illnesses [2].

Recent findings indicate the potential antidepressant effects of *G. fruticosus*. However, this plant's pharmacological and phytochemical neuroactivity potential against depression-related neuroinflammation requires validation. As a result, a comprehensive literature review was conducted, aiming to screen the phytochemistry and pharmacological properties of *G. fruticosus* to guide further studies to uncover the depression-related neuroactivity potential of this plant. The data was extracted from scientific databases, subsequently, the PRISMA guidelines and bibliometric software (VOSviewer) analysed the data to generate term maps, author associations, and country production information. The major groups of phytochemicals found in the stems, leaves, roots, and fruits of *G. fruticosus* are terpenoids, flavonoid and cardenolides glycosides within the classes of flavonoids, phenolics, pregnane glycoside, triterpenoids (e.g. betulinic acid), acetogenins, and steroidal glycoside like gomphoside and gomphotin, 3 β -taraxerol acetate. The presence of these compounds in *G. fruticosus* is greatly associated with the observed plant's anticancer, antimicrobial, antidiabetic, antimalarial, anti-inflammatory, antioxidant, antigonococcal, and antidepressant (► Fig. 1) pharmacological activities. The presence of flavonoids, steroids, and terpenoids in *G. fruticosus* confirms the potential neuroactivity of the plant [3]. This necessitates computational analysis and plant extract profiling to investigate bioactive compounds with a higher affinity for neuro-inflammation-linked receptors, thereby elucidating the mechanisms of action in subsequent *in vivo* studies.



► **Fig. 1** Chemical structure of (a) flavonoids glycosides, (b) terpenoids, and (c) cardenolide glycosides found in *Gomphocarpus* species.

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P197 Ethnopharmacological study of nine medicinal plants from Mayotte

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Mayotte, an island in the Comoros archipelago in the Indian Ocean, has rich biodiversity spanning both terrestrial and marine ecosystems. Traditional medicine remains widely practiced, relying on a diverse yet under-researched local pharmacopoeia. As part of the Integrated Innovation Hub of Mayotte (PI²M), a research focus on medicinal plants was launched to enhance the value of local biodiversity.

In 2023, a preliminary survey of 103 island residents identified 154 medicinal plant species, with 12 commonly cited for specific ailments. This led to a publication in the *Journal of Ethnopharmacology* [1]. A follow-up PhD project was initiated to assess the efficacy, safety, and quality of the most used plants. Based on criteria such as local use, endemic status, and existing pharmacological data, nine species were selected: *Coleus madagascariensis*, *Decalobanthus peltatus*, *Leptadenia madagascariensis*, *Leucas grandis*, *Mimusops comorensis*, *Paullinia pinnata*, *Phyllarthron comorense*, *Tragia furialis*, and *Vepris boiviniana*. Initial antibacterial tests on these plants showed that five had significant activity against six out of ten bacterial strains. Notably, ethanol and water extracts from *Vepris boiviniana* leaves inhibited *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* at 4 μ g/mL.

Further bio-guided studies and phytochemical analysis will isolate the active compounds, contributing to the scientific validation of Mayotte's traditional medicine and its potential inclusion in the French pharmacopoeia.

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P198 Evaluation of a multi-compound formel-Z Complex for tick infestation in 105 cats and dogs: A veterinary questionnaire study in Germany

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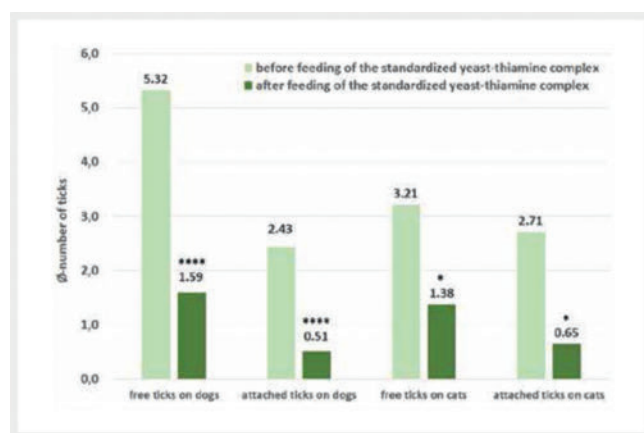
Ticks continue to spread worldwide and are becoming increasingly resistant due to the continued use of pure acaricides. As a result, natural anti-tick treatments containing repellent compounds are gaining relevance [1].

The aim of this study was to investigate the effects of the standardized thiamine-yeast complex in Formel-Z on tick infestation in dogs and cats over the course of a full tick season in a multicenter study with 23 German veterinary practices.

For this purpose, the average number of free and attached ticks was recorded through owner observation before and after the beginning of the daily admin-

istration of the natural complex (167.85 mg/kg body weight/day, as per product specifications). A total of 85 dogs and 20 cats of different breeds, all with moderately long fur, were included in this study.

The weekly number of free ticks on dogs was significantly reduced by 70.11 % after the administration of Formel-Z ($p < 0.0001$). Also, the number of attached ticks showed a significant decrease by 79.01 % ($p < 0.0001$). For cats, the weekly number of free ticks was significantly reduced by 57.01 % ($p = 0.021$), while the number of attached ticks was significantly decreased by 76.02 % after the administration of Formel-Z ($p = 0.014$; ► **Fig. 1**). In addition, a significant improvement in coat condition and skin health of dogs and cats has been reported due to the intake of Formel-Z.



► **Fig. 1** Average number of free or attached ticks on dogs or cats before and after administration of the standardized yeast-thiamine complex (Formel-Z); * $p < 0.05$; **** $p < 0.0001$

In summary, the results confirm the efficacy of the standardized natural yeast-thiamine complex in Formel-Z for the prevention of tick infestation likely due to a repellent effect, observed in both dogs and cats [2].

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P199 Antiadhesive polysaccharides against *Campylobacter jejuni*: new antivirulent strategies against early host-pathogen interaction

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The gram-negative, microaerophilic rod-shaped *Campylobacter jejuni* is the most relevant foodborne pathogen worldwide. Hygiene-related infections caused mainly by consumption of undercooked chicken meat and the resulting dysentery are long-standing problems, especially in developing countries. Inexpensive, globally available anti-adhesive natural substances are capable of inhibiting the adhesion of the intestinal pathogen, thereby reducing subsequent invasion into the host cells and preventing the infection of the host. Long-chain, low-acetylated chitosans showed anti-adhesive effects after a 2 h pre-treatment of *C. jejuni*, which reduced the number of adherent bacteria on intestinal Caco-2 cells by 65 %. They specifically inhibited the bacterial outer membrane protein Jejunilipoprotein-A (JlpA), one of the three most prominent *C. jejuni* adhesins. In contrast, chitosans with lower molecular weight and higher degree of acetylation were significantly less active [1].

Also, high-molecular extracts from fenugreek seeds (*Trigonella foenum-graecum* L.) impaired JlpA (IC_{50} 23.4 μ g/mL). In 1 h co-incubation with pathogens and host cells, the polysaccharide showed a direct reduction of the bacterial load by 50 % and also invasion into Caco-2 cells was reduced by 40 % [2].

Isolated β -glucans from *Saccharomyces cerevisiae* were able to reduce *C. jejuni* adhesion to the intestinal cells to a similar level through co-incubation. Additionally, glucans increased phagocytosis activity of RAW 264.7 macrophages to 280 % and internalization of *C. jejuni* to almost 150 %.

In summary, the polysaccharides described have considerable effects on *C. jejuni* within the infection cascade, which should lead to further development towards functional feed for livestock or as a dietary supplement for humans.

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P200 Anti-inflammatory effects of salicarinins A, B, and C from *Lythrum salicaria* L. in a porcine intestinal epithelial cell model

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Post-weaning diarrhea (PWD), primarily caused by enterotoxigenic *Escherichia coli* (ETEC), is a major gastrointestinal disorder in piglets and is characterized by intestinal inflammation. The overuse of antibiotics in PWD treatment has contributed to antimicrobial resistance, highlighting the need for alternative strategies. Hydrolysable tannin-rich plants, such as Purple loosestrife (*Lythrum salicaria* L.), are historically recognized for their anti-diarrheal properties and present a promising alternative. This study aimed to investigate the phytochemical composition of *Lythrum salicaria* L. herb aqueous extract (LSH) and assess the anti-inflammatory potential of LSH and its isolated compounds. Using a colorimetric method, we determined the total tannin content (TTC) as 270.90 ± 3.37 μ g gallic acid equivalents (GAE)/mg. Tannins represented the major polyphenol fraction in LSH. The total polyphenol content (TPC) was 279.06 ± 4.17 μ g GAE/mg. Compounds were identified using chromatographic and spectrometric techniques. Column chromatography led to the isolation of hydrolysable tannins, including vescalagin, castalagin, and salicarinins A, B, and C, enabling further investigation of their bioactivity. To model an inflammatory response similar to ETEC infection, we stimulated cells with Class B CpG oligonucleotide (ODN 2006), a synthetic DNA fragment that mimics bacterial DNA. Notably, treatment with salicarinins A, B, and C at concentrations of 10–20 μ M, 5–20 μ M, and 20 μ M, respectively, significantly suppressed IL-8 secretion in the intestinal porcine epithelial cell line IPEC-J2 without affecting its viability.

These findings suggest that LSH could help mitigate intestinal inflammation associated with ETEC-induced PWD and serve as a natural alternative to antibiotics in pig nutrition, supporting the need for further *in vivo* studies to confirm its therapeutic potential in swine nutrition.

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P201 Advancing veterinary herbal medicine through collaborative learning – dissemination in MedPlants4Vet project

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MedPlants4Vet is an international COST Action aimed at proposing a unified and simplified legislative procedure for herbal veterinary medicinal products in Europe. Its mission extends beyond legislative reform, encompassing a broader goal of enhancing knowledge and understanding of medicinal plants within the veterinary community. The project fosters collaboration among multidisciplinary working groups, including scientists and veterinary practitioners across Europe. A vital aspect of this initiative is the commitment to lifelong learning and professional development in this field.

Initial feedback from the first of several planned training courses indicates a strong demand for further education. This response highlights the need to address knowledge gaps regarding medicinal plants' use, safety, and regulatory aspects in veterinary practice. This work describes the need for dedicated training and information exchange as a key element to empower veterinarians and related professionals with the necessary knowledge to safely and effectively incorporate herbal medicine into their practices. The MedPlants4Vet project seeks to harmonize legislation and strives to build a knowledgeable, skilled community of professionals in veterinary phytotherapy. Ongoing education and cross-sector collaboration are essential for bridging existing knowledge gaps and advancing the field of veterinary herbal medicine.

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P202 Preventive effects of a standardized hydro-ethanolic extract of blackcurrant (*Ribes nigrum* L.) on high-fat diet-induced overweight in Beagle dogs

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Blackcurrant (BC) extract has been reported to exert anti-obesity effects. However, its impact on high-fat diet (HFD)-induced obesity-related disorders remains insufficiently documented. Moreover, it is not yet known whether BC extract with a composition similar to that of the whole plant (*totum*) differentially affects obesity when compared to its isolated active compounds [1]. This study evaluates the effects of a standardized hydro-ethanolic extract of blackcurrant leaves (BC-HLE) in a HFD-induced overweight dog model, comparing it to quercetin (QUE) and its combination with a *Lactobacillus*-based probiotic (PRO), consisting of three strains (*plantarum* BL2203; *fermentum* BL3413 and *rhamnosus* GG BL3113).

Twenty-four 3-year-old Beagle dogs (n = 6/group) received: HFD alone (34 % energy from beef fat), HFD + BC-HLE (12.3 mg/kg BW), HFD + BC-HLE + PRO, or HFD + QUE (0.3 mg/kg BW). Supplements were mixed into kibble and administered according to the dogs' body weight for 12 weeks.

By the end of the study, all dogs became overweight. The HFD group was associated with an increase in abdominal circumference, an elevated body condition score and signs of gut dysbiosis. The BC-HLE, whether used alone or in combination with the probiotic, mitigated the increase in abdominal circum-

ference, modulated the gut microbiome, and reduced oxidative stress. Some of these effects were also observed with QUE, though to a lesser extent. The observed efficacy may be attributed to interactions between quercetin and other phytochemicals, such as proanthocyanidins, suggesting a beneficial bio-active synergy.

These findings highlight the potential of BC-HLE as a preventive tool against canine obesity and its related disorders. However, the complex interactions between the extract and the probiotic require further investigation.

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P203 Effects of *Olea europaea* compounds on feline intestinal cells: potential application in overweight prevention in cats

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Overweight and obesity in domestic cats have significantly increased in recent years [1] with prevalence rates ranging from 11 to 63 % depending on the country [2]. Recent studies show obese cats are 2-4 times more likely to develop type 2 diabetes compared to a healthy cat [3] and up to 5 times more likely for osteoarthritis. Various therapeutic recommendations for obesity exist, such as caloric restriction or increased physical activity. Unfortunately, these methods often fail to achieve sustained weight reduction over time [4]. Among new research avenues, studies highlighted the cardiovascular benefits of Olive Leaf Extracts (OLE) due to their anti-inflammatory and antioxidant properties [5], partly attributed to their polyphenolic components oleuropein and hydroxytyrosol, as well as triterpenes like maslinic acid and oleanolic acid.

We assessed the cytotoxicity and barrier integrity effects of key OLE compounds on a feline intestinal epithelial cell line, as a first step in evaluating their anti-obesity potential.

We first evaluated the cytotoxicity (MTT assay) of oleuropein, hydroxytyrosol, maslinic acid and oleanolic acid present in OLE, on feline intestinal cells with 24h, 48h and 72h exposures. This allowed us to determine the IC₅₀, IC₅₀, IC₅₀, and IC₅₀ values for each molecule. Maslinic acid appeared to be the most cytotoxic at concentrations starting from 50 µM. We also assessed the impact of these molecules on membrane permeability by measuring TEER (Trans epithelial electrical resistance). Maslinic acid and oleanolic acid reduce this integrity at 20 µM and 50 µM, respectively. These preliminary results suggest that certain compounds from OLE exhibit concentration-dependent cytotoxic effects and negatively impact intestinal barrier function. This should be carefully considered in evaluating their safety and therapeutic potential for feline obesity management.

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P204 Indigenous knowledge-based livestock disease management practices in South Africa

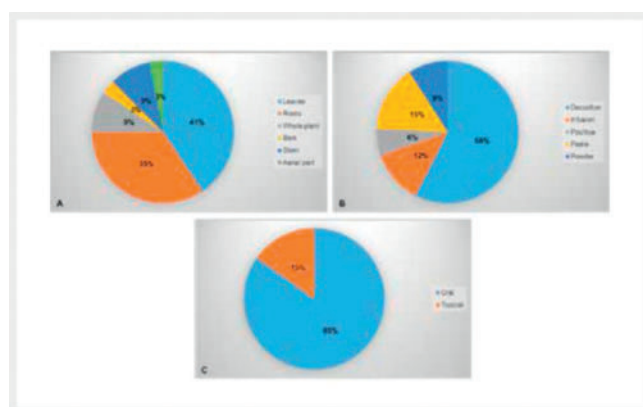
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Livestock production is essential for food security and rural livelihoods, yet infectious diseases cause significant losses [1]. Due to limited access to modern veterinary healthcare, rural South African communities rely on traditional medicinal plants for livestock healthcare [2, 3]. To better understand traditional practices used for the management of livestock diseases, a survey was conducted in the Free State Province in South Africa. Indigenous knowledge and practices were documented using structured interviews whereby the participants were traditional healers and livestock farmers. A total of 34 plant species were recorded, with commonly cited species including *Aloe* spp., *Rhamnus prinoides*, and *Cannabis sativa*. Roots (41 %) were the most frequently used plant parts, followed by the leaves (35 %) (► Fig. 1). The primary preparation methods included decoctions (58 %), pastes (15 %), and infusions (12 %). Twelve species were used for the treatment of diarrhoea, 11 species for skin ailments, and 7 species for parasitic infections. The findings highlight the importance of traditional knowledge in livestock healthcare. Furthermore, strengthening collaboration with all stakeholders including indigenous knowledge holders, particularly livestock farmers, may facilitate the integration of indigenous practices into modern veterinary science, thereby contributing to more sustainable and cost-effective livestock healthcare systems [4].



► **Fig. 1** Frequency of (A) plant parts used, (B) preparation methods and (C) administration route of plants used in ethnoveterinary medicine in the Free State Province.

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P205 Ethnoveterinary medicines for treating small ruminant ailments in zimbabwean smallholder farming systems

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Zimbabwe is a key biodiversity area in sub-Saharan Africa where communities largely rely on medicinal plants for managing livestock health. Knowledge of the plants used, and whether similar uses occur across communities, remains incomplete. This study recorded ethnoveterinary practices relating to small ruminants through semi-structured interviews with 200 informants from four districts in Zimbabwe [1]. Medicinal plants with high cultural importance and consistent botanical and therapeutic uses were identified, and the degree of similarity in medicinal plant usage between communities was analysed as an indicator of information exchange. A total of 75 medicinal plant species belonging to 35 families was documented. The foremost used medicinal plant species were from the Fabaceae family (13 species). A total of 2,703 use-reports for various diseases were categorized into nine groups and treated using these medicinal plants. Infectious and parasitic diseases received the highest number of citations (34 %). Nine plant combinations, primarily consisting of three species with comparable pharmacological properties, were reported for various ailments. *Vernonia adoensis* and *Terminalia sericea* were commonly used in these combinations. *Aloe vera* and *Lippia javanica* showed the highest botanical and therapeutic consistency, particularly against ectoparasites. Importantly, 28 new endemic medicinal plant species were reported for treatment of nine major ailments. These were mostly used for stomach disorders (8 species). Thus, ethnoveterinary medicine is the mainstay of livestock health management in resource-marginalised farming systems of Zimbabwe. However, urgent attention is warranted to break cycles of secrecy in their use and to conserve culturally important species.

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P206 Cannabidiol in veterinary medicine: Therapeutic potential and quality assurance challenges in therapeutic trend products

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Cannabidiol (CBD) has gained significant attention in veterinary medicine due to its potential therapeutic applications for conditions such as pain, anxiety, and epilepsy in companion animals. While research supports CBD's efficacy and tolerability in many cases, the rapid commercialization of CBD products has introduced challenges, including inconsistent quality and unregulated manufacturing [1, 2]. Many products lack certification, resulting in variability in active compound content and raising concerns about safety and effectiveness. This trend reflects a "fashionable" adoption of CBD without sufficient scientific validation or quality control measures. Studies highlight the need for standardized formulations and rigorous testing to ensure reliable dosing and minimize adverse effects. This work presents an overview of the described applications in veterinary medicine while addressing concerns about the quality assurance of trend products. Addressing these gaps is critical to keep CBD as a scientifically substantiated therapeutic option [3, 4].

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P207 Bistort rhizome extract supports intestinal barrier integrity in a porcine intestinal epithelial cell model

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Post-weaning diarrhea, caused by enterotoxigenic *Escherichia coli* (ETEC), is a major concern in the swine industry. With the prohibition of antibiotic use in livestock, alternative strategies are needed [1]. Condensed tannin-rich plant materials exhibit antibacterial properties and have potential as feed supplements. One such plant, Bistort rhizome (*Bistorta officinalis*), has been attributed to alleviate diarrhea. As intestinal barrier integrity is crucial for gut health, its assessment in *in vitro* models can provide valuable findings. This study investigates the phytochemical composition, antibacterial activity, and the ability of bistort rhizome aqueous extract (BR) to support intestinal barrier integrity *in vitro*. Colorimetric analyses determined polyphenol content as 235.06 ± 6.20 µg gallic acid equivalents (GAE)/mg, tannins as 214.35 ± 4.24 µg GAE/mg, and procyanidins as 148.90 ± 24.73 µg procyanidin B1/mg of BR. Twenty compounds, including procyanidins, galloyl derivatives, and flavan-3-ols, were isolated. The antibacterial potential of BR was assessed against three

common gastrointestinal pathogens, *Salmonella enterica* LT2, *Escherichia coli* ATTC25922, and ETEC O147:K89:K88, revealing no significant antibacterial activity across tested concentrations (7.88 µg/mL – 2 mg/mL). The extract exhibited no effect on ETEC adhesion to a porcine intestinal epithelial cell line (IPEC-J2). Transepithelial electrical resistance measurements of IPEC-J2 monolayers showed a significant increase at BR concentrations of 125–500 µg/mL. Our study demonstrated that while BR exhibited no antibacterial activity, it showed promising effects in enhancing intestinal barrier integrity. Given the need for antibiotic alternatives in livestock production, BR could be a potential feed supplement to support gut health, though further research is needed to confirm its efficacy *in vivo*.

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P208 Understanding complexity of psychoactive mushrooms – precision medicine approach to Parkinson's Disease

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Parkinson's Disease (PD) is a multifaceted and debilitating neurodegenerative disease with limited therapeutic options. PD is classified into familial and sporadic forms – both forms have underlying genetic alterations as gene mutations (familial; 23 genes identified) or gene expression alteration (sporadic) [1]. Familial PD contributes to PD research by driving our understanding of the underlying cellular and molecular mechanisms that underpin the cellular phenotypes exhibited in PD -alpha-synuclein aggregation (Lewy Bodies) and loss of dopaminergic neurons/dopamine release. PD patients seek alternative treatment options to alleviate their motor and non-motor symptoms, including changes in diet and use of specific supplements or plant-based controlled substances – cannabis and psychoactive mushrooms. Psychoactive mushrooms synthesise a variety of bioactive compounds from L-tryptophan into tryptamines, including psilocybin and psilocin. While their role in activating serotonin receptor is well known, recent studies show these tryptamines activate neuroplasticity pathways via TrkB receptor. As neurodegenerative diseases are underpinned by the loss of neuroplasticity and associated cell death, this project uses a precision medicine approach to investigate alignment of genetic make-up with targeted therapeutic approach using a well-defined pre-clinical model of disease. Project utilised SH-SY5Y cells overexpressing a range of different PD mutations and investigated rescue of alpha-synuclein aggregation and neurite outgrowth assays using three different mushroom extracts chosen based on traditional use (hot water) and published literature (ethanol and methanol). Preliminary results indicate phenotype rescues are mutant- and extract-specific – an outcome that allows further research into personalised therapeutics approach using controlled substances.

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P209 The effect of cloud ear mushroom extracts on UVB-induced HaCaT damages

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Global warming influences the incidence of skin damage caused by UV exposure, including sunburn, hyperpigmentation, inflammation, and skin cancer. UVB radiation is recognized as the primary contributor to oxidative stress, inflammation, DNA damage, and photoaging in the skin. Strategies for preventing and treating photoaging typically involve anti-aging, antioxidant, and anti-inflammatory approaches [1]. Notably, many natural products exhibit these properties and have been commercialized in the market. The cloud ear mushroom (*Auricularia polytricha*), an edible fungus, has been reported to possess anti-inflammatory, antioxidant, and anti-aging properties [2,3]. However, the study on the protective effects of cloud ear mushroom extracts against UVB-induced cytotoxicity in HaCaT cells remains limited. This study investigated UVB-induced cytotoxicity at various doses (15, 30, 60, and 90 mJ/cm²) in HaCaT cells. The results demonstrated that exposure to 15 mJ/cm² of UVB radiation caused significant cytotoxicity and generated reactive oxygen species (ROS) in HaCaT cells after 24 hours of post-exposure. Subsequently, HaCaT cells were exposed to UVB and treated with cloud ear mushroom extracts (hexane, ethanol, and water). Among the tested extracts, the water extract (100 µg/mL) significantly reduced oxidative stress and cell death in HaCaT cells. Furthermore, the apoptosis assay also confirmed the protective effects of the cloud ear mushroom on UVB-induced cell death. These findings suggest that the cloud ear mushroom exhibits protective effects against UVB-induced cytotoxicity in HaCaT cells and holds potential for further investigation into its molecular mechanisms related to photoaging.

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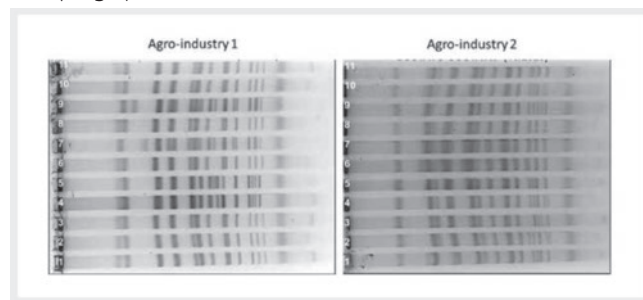
P210 Study of autochthonous yeasts isolated from cachaça-producing agro-industries with potential for use in animal feed

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DOI 10.1055/s-0045-1815111

In Brazil, agro-industries producing cachaça direct the residual yeast from the fermentation process towards animal feed. Many of these factories produce yeast on-site, isolating autochthonous microorganisms from the productive environment. However, a significant portion of these yeasts requires identification and characterization to optimize their use in feed production. This study aimed to identify and analyze the cellular growth rate of four yeasts isolated from cachaça-producing agro-industries in the Cerrado region of Minas Gerais. Samples were collected 40 days after the start of the harvest. Following plating on YPD culture medium, the colonies underwent karyotyping analysis [1], selection, and multiplication to obtain 2g of biomass. Subsequently, the microorganisms were transferred to a 7L bench-top bioreactor, where fermentation was performed using sugarcane juice as the substrate, evaluating the budding index [2]. It was observed that industrial yeast cultures contain more than one dominant strain, with two isolates from industry 1 (CM-01 and CM-02) and three from industry 2 (CM-05, CM-06, and CM-07). CM-01 demonstrated the highest budding rate at the beginning of the fermentation, while CM-02 and CM-03 exhibited higher rates towards the end of the process. In conclusion, cachaça-producing agro-industries in Brazil exhibit a diversity of autochthonous yeasts involved in the fermentation process, with potential applications in animal nutrition. The cellular growth and budding characteristics of the analyzed yeasts varied throughout fermentation, highlighting

each microorganism's specific relevance to the production process. Identifying and characterizing these yeasts, helps to optimize their use, promoting greater efficiency in feed production and sustainable management of agro-industrial residues (► Fig. 1).



► **Fig. 1** Karyotyping results of yeasts from two cachaça-producing agro-industries.

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P211 Metabolic characterization and skin-protective effects of a *Penicillium chrysogenum* extract against aging, UV-induced damage, and inflammation in HaCaT cells

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DOI 10.1055/s-0045-1815112

Penicillium chrysogenum is a well-known saprophytic fungus recognized for its antibiotic production [1]. This microorganism can be found in a variety of aquatic and terrestrial environments, and its fermentation activity is important to the pharmaceutical sector, especially for penicillin extraction [2]. In this study, a strain of *P. chrysogenum* was isolated from shallow marine sediments of Amendolara (Calabria, Italy). A *n*-butanolic extract (R_{Bu}OH) from the mycelium and culture medium was analyzed using UHPLC-HR-Orbitrap/ESI-MS, leading to the identification of several cyclopeptides. The potential of the R_{Bu}OH extract to protect the skin from i) oxidative aging, ii) photoaging due to UV-A (20 J/cm²) and UV-B (40 mJ/cm²) radiation, and iii) inflammation caused by lipopolysaccharides (LPS) was assessed *in vitro* using HaCaT cells (human keratinocytes), considering the anti-aging and anti-inflammatory properties described for several natural cyclopeptides [3,4].

Cytotoxicity assays showed no significant toxicity up to 10 µg/mL. The extract protected Ha-CaT cells from H₂O₂-induced oxidative damage (600 µM) at 0.1–10 µg/mL. Its anti-aging activity was confirmed by a significant reduction in cellular senescence, measured by SA-β-Gal activity in cells treated with H₂O₂

(200 μ M). The extract was also tested before and after UV-A and UV-B exposure, showing a reduction of UV-induced damage only when applied afterwards. Finally, the RBUOH extract showed significant protection against the inflammatory response induced in HaCaT cells by LPS (10 μ g/mL).

In conclusion, these findings suggest a potential use of the RBUOH extract from *P. chrysogenum* in skincare formulations in view of its antioxidant and anti-inflammatory effects.

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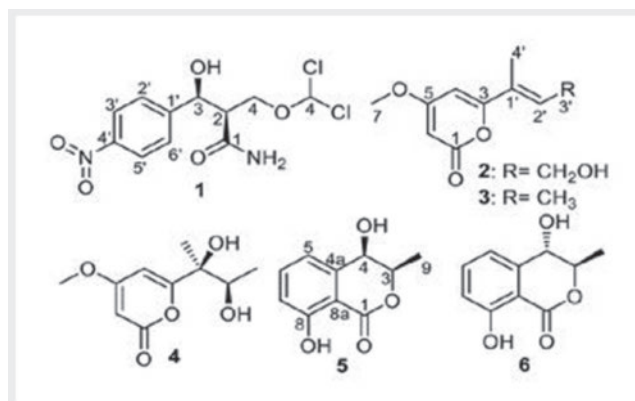
P212 Acrodontiolamide, a rare chlorinated fungal metabolite, and five bioactive α -Pyrone from an endophytic *Nigrospora* sp.

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Endophytic microorganisms are recognized as potential sources of novel bioactive compounds for pharmaceutical applications. This study [1] investigated the secondary metabolites produced by an endophytic *Nigrospora* sp. isolated from the Nigerian plant, *Gongronema latifolium*. Standard procedures were used for fungal isolation, taxonomic identification, fermentation, and extraction of secondary metabolites. Chromatographic and spectroscopic techniques were used to isolate and characterize compounds from the fungal extract. The fungal extract yielded a rare chlorinated metabolite, acrodontiolamide (1), and five α -pyrone derivatives: hydroxypestalopyrone (2), pestalopyrone (3), pestalotiopyrone D (4), *cis*-4-hydroxymellein (5), and *trans*-4-hydroxymellein (6) (► Fig. 1). Acrodontiolamide, like many fungal secondary metabolites, has a nitro group attached to an aromatic ring. However, unlike typical fungal secondary metabolites where chlorination occurs on the aromatic ring, chlorination in acrodontiolamide occurs on its aliphatic moiety. Few natural product compounds have been reported with monochlorination on the aliphatic C3 chain, making this type of halogenation uncommon. Notably, this study marks only the second report of acrodontiolamide in nature, following its initial discovery in 1993 [2]. Acrodontiolamide and the five α -pyrone derivatives have been reported to possess various pharmacological activities, including antimicrobial, cytotoxic, anticancer, and antimalarial properties [2–5], highlighting their potential in drug development and the value of endophytic microorganisms as sources of new bioactive compounds.



► **Fig. 1** Acrodontiolamide (1) and five α -pyrone derivatives: hydroxypestalopyrone (2), pestalopyrone (3), pestalotiopyrone D (4), *cis*-4-hydroxymellein (5), and *trans*-4-hydroxymellein (6) isolated from an endophytic *Nigrospora* sp.

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P213 Co-culture of the fungus *Fomitopsis betulina* with *E. coli* on solid media for the detection of antibacterial candidates: Deciphering the bacterial-fungus interaction

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Antimicrobial resistance represents an increasing worldwide threat [1]. In this scenario, innovation in antibacterial compound sourcing, especially from fungi that represent bio-sourced and unexplored alternatives to synthetic compounds, is needed to increase the treatment success of infections. Most of the described antibacterial metabolites with fungal origins (penicilline derivatives, cephalosporines) are issued from the Ascomycota division (*Penicillium* sp., *Acremonium* sp.). Antibacterial potential of Basidiomycota, the sister group of Ascomycota, is well known, but secondary metabolism of these fungi is expressed only in specific, cryptic conditions. Secondary metabolite biosynthetic pathways are thus poorly known in Basidiomycota.

In this context, co-culture experiments on solid media were performed to evidence the biosynthesis of antibacterial metabolites from the fungus *Fomitopsis betulina* (Basidiomycota) in the presence of the bacteria *E. coli*. An antibacterial activity, determined by an *E. coli* growth inhibition (inhibition zone) was

observed. Using mass spectrometry metabolomic analyses combined with targeted purification of metabolites, the objective of this study was to highlight and characterize antibacterial candidates.

Only one candidate, constitutive of the fungus, was accumulated at the inhibition zone according to the metabolomic results. The compound of interest, purified from the fungus secretome and characterized by 1D and 2D NMR, appears to be a calcium complex of three units of Sumiki's acid, but did not exert any direct antibacterial activity against *E. coli*. No other antibacterial metabolites was detected through this MS-based metabolomic study.

Interrogations remain regarding the mechanisms of growth inhibition and the correlative accumulation of this chelating agent in the inhibition zone.

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P214 The potential of polypore extracts to modulate angiogenesis and inflammation: Insights from *in vitro* screening on endothelial cell proliferation, migration, and leukocyte recruitment

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Polypores have a rich history in traditional medicine and significantly contribute to the ethnopharmacological alimentarium across diverse cultures. These fungi produce diverse specialized metabolites and have supposed antitumoral, lipid-lowering, antiviral, and either immune-stimulating or anti-inflammatory activities [1].

We screened over 80 polypore extracts and fractions against various functional *in vitro* assays related to angiogenesis and inflammation, including endothelial cell proliferation, migration, and leukocyte recruitment to endothelial cells. Several ethanolic extracts, notably of *Inonotus obliquus*, *Pseudoinonotus dryadeus*, *Laricifomes officinalis*, and *Piptoporus betulinus*, inhibited the proliferation of human umbilical vein endothelial cells (HUVECs), suggesting potential anti-angiogenic activity. MS networking identified lanostane-type triterpenoids as contributors to these effects. Given its promising bioactivity, *Inonotus obliquus* was selected for further mycochemical investigation. This led to the identification of the lanostane-type triterpenes lanosterol, inotodiol, and inonotsutrial A as active principles, with their biological effects showing some correlation with lipophilicity. Conversely, only a few extracts inhibited leukocyte recruitment to endothelial cells, suggesting limited anti-inflammatory activity. This contrasts with other reported immunomodulatory effects of polypores, such as inhibition of cytokine release and inhibition of NF- κ B in LPS stimulated leukocytes [2].

Our findings highlight the potential of polypores to modulate endothelial cell proliferation, suggesting some potential to inhibit angiogenesis, which aligns with their ethnomedicinal use as antitumoral agents. However, their influence on inflammation appears selective, warranting further investigation into their immunomodulatory properties.

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P215 Unveiling the hidden chemistry of Antarctic fungi: Metabolomic Insights from OSMAC-based diversification

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The Antarctic environment harbors a unique and chemically underexplored fungal diversity with promising biosynthetic potential [1]. Exploring these cold-adapted fungi contributes to Sustainable Development Goal (SDG) 15, promoting the sustainable use of terrestrial ecosystems, and SDG 9, by fostering innovation through novel natural products. Unlocking their secondary metabolites may lead to new biotechnological applications while reinforcing the value of preserving fragile polar ecosystems. In this study, the OSMAC (One Strain-Many Compounds) approach was employed to investigate the chemical diversification of six Antarctic fungal strains: *Penicillium terrigenum*, *Epicoccum nigrum*, *Periconia* sp., *Penicillium chrysogenum*, *Antarctomyces psychrotrophicus*, and *Rhodotorula* sp. The marine-derived fungi *P. terrigenum*, *E. nigrum*, and *Periconia* sp. were cultivated in five different media: rice, malt, potato dextrose, and Sabouraud broths supplemented with sea salt, as well as in marine broth. In contrast, *P. chrysogenum*, *A. psychrotrophicus*, and *Rhodotorula* sp. were grown in potato dextrose, malt, and Sabouraud broths without sea salt. Fermentations were performed in triplicate for 14–28 days and extracted with ethyl acetate (3 $\times$). Extracts were subjected to chemical analyses using ¹H NMR and HPLC-DAD. Multivariate analysis (PCA) of the ¹H NMR spectra unveiled shifts in metabolite profiles driven by culture conditions. These findings underscore the role of media composition in modulating fungal secondary metabolism. Distinct molecular signatures emerged across different culture media, revealing how subtle environmental tweaks can reshape chemical output. In *E. nigrum*, salinity enhanced the production of epicorazine A, illustrating how the OSMAC approach can effectively awaken silent biosynthetic pathways and expand chemical diversity.

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P216 Studies on antioxidant, antimicrobial and cytotoxic activity of *Phellinus nigricans* mushroom extracts

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The genus *Phellinus* s.l. belongs to the family *Hymenochaetaceae* and includes over 220 described taxa worldwide, including 35 taxa in Europe [1, 2]. Some

species belonging to this genus have been traditionally used in Chinese medicine and have proven health-promoting effects [3]. As part of the research project, above 30 species of fungi from the genus *Phellinus* were studied. Fruit bodies of fungi of the genus *Phellinus* were collected in the Białowieża Forest, Romnicka Forest, the coastal areas of Poland, as well as from Portugal and Italy. Methanol extraction of dried mushroom fruit bodies was performed. Preliminary screening of antimicrobial activity was performed on selected bacterial strains and antioxidant activity tests were performed using DPPH and ABTS radical tests. Mushroom extracts with the highest antimicrobial and/or antioxidant activity were selected and subjected to further tests. The elemental composition of the extracts was determined, the analysis of active compounds (GCMS), FTIR spectroscopic analysis and thermal decomposition (TG/DTG) were performed. Antimicrobial activity of the extracts on multidrug-resistant bacterial strains was also tested. The paper presents the results of studies on the species *Phellinus nigricans*. Fruiting bodies living on different hosts were examined (*Betula Pendula*, *Betula Pubescens* and *Fagus Sylvatica*). The content of natural compounds in the extracts and the biological activity of the species were compared depending on the host on which the fungus lived. Cytotoxicity studies were performed on a normal human fibroblast cell line (CRL) and on an immortalized cell line derived from human epidermal cells (HaCaT).

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P217 Bioactivity assessment of european *Phellinus* spp.: Antibacterial and antioxidant potential

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Phellinus is an important genus within the Hymenochaetaceae family, comprising around 220 species worldwide, and 35 taxa occurring in Europe [1, 2]. Taxonomically, *Phellinus* is a complex genus exhibiting rich species diversity across various habitats, including both temperate and tropical forests [3]. The genus has undergone extensive taxonomic revisions, with recent molecular phylogenetic studies providing deeper insights into the evolutionary relationships

within this group [4]. In Europe, several *Phellinus* species are noted for their ecological and medicinal significance, with some having been used in traditional medicine for centuries [5].

This study aimed to perform a comparative analysis of 16 *Phellinus* species, collected from Poland, Macedonia, Italy, Portugal, and Kosovo. A series of microbiological tests (MIC) and antioxidant assays (DPPH, ABTS, lipid peroxidation) were conducted on all samples. Subsequently, chemical composition analyses (GC-MS) were performed on the 10 species with the highest biological activity identified.

The results clearly demonstrate the significant biological activity of the selected *Phellinus* species, particularly their antimicrobial and antioxidant properties, highlighting their potential for health-related applications and suggesting that further studies of their bioactive compounds are recommended.

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P218 Diastereoisomeric biflavonoids and a cleaved biflavonoid from *Ochna rhizomatosa*

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Biflavonoids are structurally diverse secondary metabolites with biological activity of high relevance, such as anticancer, antibacterial, antioxidant and anti-inflammatory, among others [1, 2]. In this study, we report the isolation of two diastereoisomeric biflavonoids (Fig. 1), alongside a newly identified cleaved biflavonoid and 13 known biflavonoids, from the stem bark of *Ochna rhizomatosa*. Structure elucidation was performed using advanced spectroscopic techniques, including NMR spectroscopy, HR-ESIMS, and circular dichroism (CD) spectroscopy.

The newly identified diastereoisomer was distinguished by its unique stereochemistry at the interflavonoid linkage, confirmed by CD spectroscopy [3]. Additionally, the cleaved biflavonoid exhibited an unusual fragmentation pattern, suggesting enzymatic or oxidative cleavage.

Bioactivity assays revealed that the new diastereoisomer exhibited selective cytotoxicity against human cancer cell line KB-3-1, while the known diastereoisomer showed moderate antibacterial effects. The cleaved biflavonoid demonstrated significant cytotoxic activity. These findings highlight the structural diversity and pharmacological relevance of biflavonoids, emphasizing the impact of stereochemistry and cleavage modifications on biological activity.

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P219 Comparative analysis of bioactive phenolic compounds from flower buds and leaves of *M. officinalis* var. *biloba* Rehder & Wilson

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Magnolia officinalis var. *biloba* Rehder & Wilson, a perennial deciduous tree of the genus *Magnolia*, is endemic to southern China [1]. Continuing previous research [2], dried flower buds (MOBK) and leaves (MOBL) of *M. officinalis* var. *biloba* collected from the Botanical Garden in Powsin (Poland) were subjected to ultrasound-assisted extraction using 30, 50 and 70 % (V/V) EtOH as extraction solvents. Herbal preparations were analysed using coupled chromatographic (RP-LC), spectroscopic (PDA) and mass spectrometric (QTOF/ESI-MS/MS) techniques. The LC/MS investigation showed different quantitative and qualitative profiles of the main phenolic compounds, including phenolic acids, phenylethanoids, flavonols, lignans and neolignans. Their total content, determined as gallic acid equivalent (GAE) in spectrophotometric (Vis) studies, increased significantly with the elution strength of the extraction solvent and was highest in extracts obtained with 70 % EtOH (49.92 and 34.32 mg GAE/g of dry wt, for MOBK and MOBL, respectively). Interestingly, the antiradical capacity of the above-mentioned extracts, as assessed by the DPPH₁ assay, was strongly correlated with the total lignans and neolignans and phenolic acid content, which was about two (for lignans) and four times (for phenolic acids) higher in MOBL than MOBK. As a result, the former extract exhibited higher antiradical activity (IC₅₀ ~ 10.15 µg/mL) than the latter (IC₅₀ ~ 24.76 µg/mL).

Our study showed a close relationship between the type of plant material and the content of particular phenolic compounds, especially phenylethanoids, which are synthesised mainly in flower buds, whereas higher amounts of phenolic acids, lignans and neolignans were found in leaf extracts. Differences in the distribution of individual phenolic components in plant organs may affect their biological properties.

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P220 The induction effect of *Anastatica hierochuntica*, *Cassia alexandrina* and *Rumex vesicarius* plant aqueous extracts on cytochrome P-450 enzyme gene expression

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Cytochrome P-450 (CYP) enzymes are among the main metabolic systems for drug metabolism with 1A2, 2B6, 2C8, 2C9, 2C19 and 3A4 being among the most crucial clinically. This study explores if consumption of popular Saudi Arabian herbs of *Anastatica hierochuntica*, *Cassia alexandrina* and *Rumex vesicarius* modulate the activity of CYPs in HepG2 cells. Both boiled and sonicated aqueous extracts were prepared to mimic common consumption methods. The endotoxin contamination level and HepG2 cell viability were assessed using LAL and CellTiter-Glo kits, respectively, to identify non-toxic extract concentrations for CYP gene expression studies. The gene expression was measured using RT q-PCR. Pregnane X receptor (PXR) ligands were detected using TR-FRET competitive binding assay. The IC₅₀ values for extracts ranged from 78.1 to > 1000 µg/mL, thus a concentration of 20 µg/mL was chosen for CYP gene expression studies. The results show that *Cassia alexandrina* sonicated aqueous extract induced 1A2, 2B6, 2C9, 2C19 and 3A4 up to 2-fold, and 2C8 up to 4-fold change. *Rumex vesicarius* sonicated extract induced 2C8 and 2C9 up to 2-fold change. *Anastatica hierochuntica* boiled and sonicated extracts induced all CYPs except 3A4 up to 2-fold. The receptor binding assay suggest the induction by *Cassia alexandrina* may be mediated through binding of its constituent(s) to PXR while *Anastatica hierochuntica* and *Rumex vesicarius* may act via the activation of constitutive androstane receptor. These findings indicate the potential for serious herb-drug interactions if these highly consumed plants are co-administered with narrow therapeutic index drugs mainly metabolized by the studied CYP enzymes.

P221 Empirical modeling of ¹JCH coupling constants using genetic programming

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One-bond heteronuclear coupling constants (¹JCH) are valuable for the configurational and conformational analysis of organic molecules. [1–3] For instance, they are employed to differentiate α- and β-anomeric protons in carbohydrates and to distinguish equatorial from axial protons in cyclohexane rings via the Perlin effect. However, the prediction of these NMR parameters by Density Functional Theory (DFT), useful for comparing them with experimental values, is limited by the high computational cost.

To offer a more accessible alternative, we extended a previously developed computer-aided strategy, originally applied to the prediction of one-bond carbon-carbon coupling constants (¹JCC), to the empirical prediction of ¹JCH values.

Using genetic programming (via gplearn) and scikit-learn [4], we generated mathematical models capable of accurately predicting experimental scalar coupling constants with reasonable computational costs and times. In this case, a dataset of 148 ¹JCH values from diverse organic molecules was used to train the models. To enhance predictive performance, we integrated both NMR and structural descriptors, e.g., chemical shifts and bond angles. This multi-parametric approach significantly improved accuracy, achieving an R² value of 0.96, while greatly reducing computational cost compared to DFT.

Beyond performance, the method is fast and interpretable, making it suitable for integration into stereochemical elucidation workflows. Future efforts will focus on validating this model on independent test sets. Additionally, comparisons among the values predicted by our developed empirical strategy, those DFT-predicted and experimental ones, will assess accuracy and generalizability. This approach will also be tested on selected case studies to further verify its utility in practical applications.

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P222 Investigating the phytochemical content and anticancer properties of the endemic plant of Cyprus *Bosea cypria*

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Bosea cypria, locally known on the island as "zoulatzia," is an evergreen endemic shrub that grows in rocky terrains, up to 600 m altitude. Taxonomically, it belongs to the Amaranthaceae family and is one of the only three species (all endemic) of the *Bosea* genus found worldwide. In this study, the phytochemical composition of different botanical parts of the plant (leaves, stems, fruits) was examined, along with its variation based on collection site (Potamos Liopetriou 15 m, Pelathousa 300 m, Kritou Terra 430 m). Moreover, the anticancer properties of selected extracts were investigated using the MTT assay on two breast cancer lines (MDA-MB-231 and MCF-7). Phytochemical analysis revealed that both the plant part and, secondarily, the collection site determine the phytochemical composition and antioxidant capacity. Notably, the interaction between these two factors significantly influences the phytochemical content of the extracts. Extracts from plant samples collected at Potamos Liopetriou and Pelathousa exhibited higher antioxidant capacity and flavonoid content but lower tannin concentrations compared to those from Kritou Terra. Leaves showed the highest concentrations in all measured parameters, followed by stems and fruits in descending order. Regarding the MTT assay results, the two selected extracts from leaves and stems revealed dose- and time-dependent effects on both cancer cell lines, with a pronounced reduction in cell viability observed in the more sensitive cell line (MCF-7) at both 24 and 48 hours of incubation (reduction >40%, $p < 0.05$). This study concludes that *Bosea cypria* represents a significant reservoir of phytochemicals, with leaf extracts demonstrating promising anticancer potential.

P223 Phytochemical composition and anticancer potential of the endemic gold oak of Cyprus *Quercus alnifolia*

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Quercus alnifolia, is an evergreen endemic oak, grows in the Troodos peninsula. Leaf dichromatism is a notable characteristic of this species, with young leaves showing a dark green adaxial and gold abaxial, while mature leaves have a brown abaxial (Fig. 1). The aim of the study was to investigate the phytochemical composition of young and mature leaves, along with its variation based on season (summer and winter) and collection site (three distinct altitudinal sites). In addition, the phytochemical composition and antioxidant activity of other botanical parts, such as acorns and bark, were determined. Subsequently, the anticancer properties of selected leaf extracts were examined using the MTT assay on breast (MDA-MB-231 and MCF-7), colon (HT29, HCT116), and lung cancer cell lines (H1975, A549). Additionally, the potential phytotoxic effects of the extracts were examined on a normal breast cell line MCF-12F. Phytochemical analysis revealed that leaf age is the predominant factor influencing phytochemical composition, particularly concerning total phenolics and antioxidant capacity, which were significantly higher in young compared to mature leaves. The collection season influenced flavonoid and tannin concentrations. Among the other botanical parts, fruits and young bark exhibited promising phytochemical composition. As for anti-cancer properties, the selected young leaf extracts showed a significant dose- and time-dependent reduction of cell viability in all cancer cell lines examined, while its impact on MCF-12F cells was limited. Concluding, this study highlights the significance of *Q. alnifolia* as a rich source of phytochemical compounds and emphasizes the potential of leaf extracts as anticancer agents.

P224 Isolation, structure elucidation and anti-inflammatory testing of sesquiterpene lactones and flavonoids from *Achillea nobilis* in an ICAM-1 *in vitro* assay

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The genus *Achillea* contains about 130 species and in addition to well-known *Achillea millefolium* several others are also used for the treatment of wounds, inflammations, spasmodic diseases and dyspepsia [1, 2]. The ethanolic extract of *Achillea nobilis* showed anti-inflammatory effects and the species contains several secondary metabolites such as flavonoids, sesquiterpene lactones (STL) and essential oil [3, 4].

Since the chemical profile of *A. nobilis* is not known in detail, it was worked on the isolation and structure elucidation of compounds of its DCM-extract followed by anti-inflammatory testing. Two known monomeric STL, namely 5 α ,10 α -dihydroxy-1 α H-dehydroleucodine (1) and 3-oxo-1 β -hydroxy-5 α H,6 β H,7 α H-eudesma-4(15),11(13)-dien-6,12-olide (2), were isolated together with one new dimeric STL (3) (Fig.1). Additionally, two known polymethoxylated flavonoids were obtained and elucidated as casticin (4) and centaureidin (5). In order to determine the anti-inflammatory effect of 1–3 and

5, they have been tested in a ICAM-1 *in vitro* assay in concentrations between 1–75 μM . [5] Thereby, the ICAM-1 expression after stimulation of a human endothelial cell line (HMEC-1) with TNF α was significantly reduced by all isolates. The most active substance was casticin with an IC₅₀ value of 11.0 μM , while the STL showed slightly weaker but similar effects with IC₅₀-values between 13.9–16.9 μM . These results show the promising potential of the isolated secondary metabolites for use in inflammation. Further research of the chemical profile of *A. nobilis* is in progress.

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P226 Effects of plant-based extracellular vesicles from St. John's wort and Calendula on Wound healing

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Plant-based extracellular vesicles (EVs) are emerging as a promising avenue for biomedical applications, showcasing their potential in drug delivery, biomarker discovery, and intercellular communication due to their unique biocompatibility and ability to carry diverse molecular cargo. This study investigates the potential of plant-based EVs as innovative active ingredients for wound healing, focusing on their effects on cellular processes involved in tissue repair. We evaluated two traditional herbal treatments—St. John's wort (*Hypericum perforatum*) and Calendula (*Calendula officinalis*)—to isolate their EVs and assess their impact on inflammation, cell migration, and tissue remodelling using keratinocytes and dermal fibroblast as cell models. Key findings indicate that while both herbal compounds in doses traditional used in topical management of wounds (St. John's wort: $c = 3 \text{ mg/cm}^2$ with $5.22 \times 10^9 \text{ EVs/cm}^2$; Calendula: $c = 3 \text{ mg/cm}^2$ with $3.51 \times 10^8 \text{ EVs/cm}^2$) and their derived EVs purely in corresponding concentrations modulated inflammatory responses and cellular activities. Their effects varied depending on the cell type. Specifically, Calendula EVs ($c = 3.51 \times 10^8 \text{ EVs/cm}^2$) inhibited keratinocyte migration, potentially delaying epidermal reconstitution, while enhancing dermal fibroblast migration, suggesting a dual role in extracellular matrix modulation. Furthermore, both herbal extracts ($c = 3 \text{ mg/cm}^2$) and their EVs (St. John's wort: $c = 5.22 \times 10^9 \text{ EVs/cm}^2$; Calendula: $c = 3.51 \times 10^8 \text{ EVs/cm}^2$) inhibited alpha-smooth muscle actin (α -SMA) expression, indicating a reduction in myofibroblast differentiation, which could minimize scar formation in chronic wounds. Overall, this research highlights the therapeutic potential of plant-derived EVs in wound healing, warranting further exploration into their mechanisms of action and clinical applications.

P227 Phytochemical characterization and cytotoxic activity potentials of isolated compounds and extracts from Propolis

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Propolis is a natural resinous substance collected by honeybees from various plant sources and mixed with wax and enzymes. It has attracted significant scientific interest due to its broad spectrum of biological activities, including antimicrobial, anti-inflammatory, antioxidant, and cytotoxic effects [1, 2]. These properties make propolis an important candidate for pharmaceutical research. However, its chemical composition is highly dependent on geographic origin and local flora, which complicates standardization [3]. Therefore, isolating and identifying the active compounds in a given sample is essential to understanding its therapeutic potential. In this study, a phytochemical investigation was conducted on propolis samples collected from Kelkit (Gumushane, Türkiye). Four natural compounds were successfully isolated using further chromatographic techniques, and their structures were elucidated using ¹H and ¹³C NMR spectroscopy. These compounds were identified as pinocembrin, 7-O-methyl-datiscetin, galangin, and tectochrysin. The cytotoxic effects of the 70 % ethanol extract and the isolated compounds of propolis were assessed using a MTT assay. The evaluation was conducted on SK-BR-3 (breast adenocarcinoma), OE33 (esophageal adenocarcinoma), ACC-201 (gastric adenocarcinoma), and CCD-34Lu (normal lung fibroblasts) cell lines. Cells were incubated for 24 hours and treated with concentrations of 0.5, 5, and 50 $\mu\text{g/mL}$ for 48 hours. Doxorubicin was used as the positive control [4]. The most notable cytotoxic effect was observed for the ethanol extract, with an IC₅₀ value of $38.33 \pm 5.61 \mu\text{g/mL}$ against the OE33 cell line. These results suggest that the cytotoxic effects observed in the ethanol extract may be attributed to a synergistic interaction between its bioactive compounds.

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P228 Synthesis and *in vitro* evaluation of oleanolic acid derivatives with enhanced neuroprotective and cytotoxic activities

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Oleanolic acid, a naturally occurring triterpenoid compound, has garnered significant attention for its potential therapeutic benefits [1, 2]. In this study, oleanolic acid, isolated from *Scabiosa pseudograminifolia*, was used as the starting material for semisynthetic modifications to enhance neuroprotective activity and cytotoxic effects. Three derivatives were synthesized through amidation reactions em-

ploying 3-(1*H*-tetrazol-5-yl)aniline (S1), 4-(1*H*-tetrazol-5-yl)aniline (S2), and 4-bromobenzylamine (S3), with yields of 68% for S1, 72% for S2, and 56% for S3. The neuroprotective potential of these compounds was evaluated using the MTT assay on SH-SY5Y human neuroblastoma cells subjected to hydrogen peroxide-induced oxidative stress at concentrations of 0.5, 1, 5, 10, 50, and 100 µg/mL. At the lowest concentration (0.5 µg/mL; 0.83 µM for S1 and S2, 0.80 µM for S3), S3 exhibited the highest cell viability (133.88 ± 2.47 , $P < 0.0001$), followed by S1 (121.70 ± 2.60 , $P < 0.0001$) and S2 (112.14 ± 1.65 , $P < 0.001$), while oleanolic acid showed 115.35 ± 4.90 ($P < 0.0001$). Similar trends were observed at 5 µg/mL and 50 µg/mL, with S3 consistently providing superior protection. In parallel, cytotoxicity was assessed on HEK-293 cells and various cancer cell lines (PC-3, A549, MDA-MB-231, PANC-1, SH-SY5Y) [3]. Notably, S2 demonstrated enhanced cytotoxic activity against A549, MDA-MB-231, and PANC-1 cells with IC_{50} values of 57.21 ± 4.40 µM, 131.78 ± 6.83 µM, and 81.87 ± 4.65 µM, respectively, compared to higher values for the parent compound. These findings indicate that structural modifications of oleanolic acid can improve both neuroprotective and cytotoxic profiles, warranting further mechanistic and *in vivo* investigations.

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P229 Anthocyanin-enriched extracts and geranylgeraniol cytoprotective effect on bisphosphonate-treated epithelial cells

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Bisphosphonates are widely used drugs for the treatment of osteoporosis and bone metastases. Despite bisphosphonate's clinical importance, chronic use is associated with drug-induced osteonecrosis of the jaw (MONJ). In the search for alternatives treatments for this condition, natural products arise as a promising source of new leads. For example, geranylgeraniol (GG), a diterpene found in several species such as annatto (*Bixa orellana* L.), has been evaluated to manage MONJ [1]. Furthermore, anthocyanin-enriched species such as *Fridericia chica* (Bonpl.) L. G. Lohmann and *Fragaria ananassa* Duch. are promising sources due to their antioxidant, anti-inflammatory, and wound healing effects [2, 3]. Herein we report our ongoing research [4, 5], evaluating the cytoprotective effects of GG-enriched fraction from *B. orellana*, *F. chica* (leaves), and *F. ananassa* (fruits and leaves) extracts on zoledronic acid (ZA)-treated human immortalized keratinocytes (HaCaT). HaCaT cells were exposed to ZA (5 µM) for 24 h before treatment with the selected extracts for an additional 24 h (curative protocol). The sulforhodamine B assay assessed cell viability. Considering untreated and unchallenged cells as 100% viable cells, ZA-treated HaCaT showed $91.3 \pm 2.6\%$ viable cells. The best results were observed for GG-enriched fraction (15 µg/mL, $107.6 \pm 3.5\%$), *F. ananassa* fruit extract (25 µg/mL, $100.7 \pm 0.6\%$), *F. chica* leaf extract (5 µg/mL, $96.4 \pm 2.0\%$), and *F. ananassa* leaf extract (25 µg/mL, $92.7 \pm 5.3\%$). Further studies will evaluate the association of these extracts to test synergic effects.

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P230 Gnaphalolide A, a new chalcone, along with phenolic compounds in *Pseudognaphalium affine* exhibited noteworthy effects on xanthine oxidase

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Cudweed is a common name of a numbers of species belonging to genus *Gnaphalium* and *Pseudognaphalium*, Asteraceae family. Recent literature has reported a potentially inhibitory effect on xanthine oxidase (XO-i) of *Pseudognaphalium affine*. In the course of study on species of cudweed grown in Vietnam, this work compared XO-i activity of two species *P. affine* and *G. polycaulon*, and investigated on phytochemicals from promising fractions. *In vitro* XO-i activity of methanolic extracts from different parts of use of the two plants was compared. Amongst tested samples, the methanolic extract of the flowers of *P. affine* exhibited potential activity (IC_{50} of 35.34 µg/mL, comparing to 1.41 µg/mL of allopurinol). XO inhibitory activity-guided phytochemical analyse of the flowers of *P. affine* led to isolation of seventeen compounds. Among them, a new chalcone (1) was identified, together with sixteen known compounds, including one chalcone (2), two flavones (3, 4), four flavonols (5–8), two flavanones (9, 10), three caffeic acid derivatives (11–13), and four miscellaneous compounds (14–17). The structure of 1 was determined on the basis of its spectroscopic data to be 4,2',4'-trihydroxy-6'-methoxychalcone-4'-O-β-D-(6''(E)-caffeoyl)-glucoside, and it was named gnaphalolide A. Twelve of seventeen compounds, including the new chalcone, exhibited noteworthy inhibition on XO (with IC_{50} value of 7.41–104.42 µM, comparing to 1.13 µM of allopurinol). Our findings unveiled the potential of phytoconstituents from *P. affine* grown in Vietnam to alleviate XO activity in related disorders [1–5].

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P231 Modulation of *Pseudomonas aeruginosa* biofilm architecture using natural carotenoids: An innovative approach to enhance antibiotic efficacy

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Antibiotic resistance is one of the most serious global health threats. Among the priority pathogens, *Pseudomonas aeruginosa* stands out due to its multi-drug resistance and ability to form protective biofilms, severely limiting the efficacy of conventional treatments [1]. This study investigates the potential of carotenoid-rich natural products to modulate the biofilm architecture of *Pseudomonas aeruginosa* PAO1 WT, with the goal of enhancing antibiotic penetration and efficacy. Based on their content, carotenoid fraction extracts of *Solanum lycopersicum* L., *Ipomoea batatas* L., and *Cucurbita moschata* Duchesne, were tested at various concentrations. Their effects on biofilm formation and virulence factors were assessed using scanning electron microscopy, digital microscopy, motility assays, and quantification of pyocyanin production, alongside chemical profiling by HPTLC and GCMS. Preliminary results highlight promising anti-biofilm activity from several extracts, supporting a potential use of these natural compounds as adjuvants to antibiotic therapy.

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P232 Metabolite Profiling of “Carciofo di Paestum” PGI and “Bianco di Pertosa” (*Cynara cardunculus* subsp. *scolymus*) leaves by ¹H NMR and HRMS-Based Metabolomics

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The globe artichoke (*Cynara cardunculus* subsp. *scolymus*, Asteraceae) is a perennial plant native to the Mediterranean. It is traditionally cultivated for its large, immature flower heads (capitula), used as a vegetable in Mediterranean dishes. These heads are consumed fresh or processed into frozen, cooked, canned, or oil-preserved products. The artichoke is a functional food due to its high content of minerals, bioactive phenolic compounds, and inulin [1]. Artichoke cultivation and processing generate significant waste (80 %-85 % of total biomass), mainly leaves. This study evaluates the potential of artichoke leaves from two Campania varieties, ‘Bianco di Pertosa’ and ‘Carciofo di Paestum’ PGI, focusing on bioactive compounds [2].

LC-ESI/HRMSMS analysis of hydralcholic extract (EtOH:H₂O 75:25) of “Bianco di Pertosa” and “Carciofo di Paestum” leaves revealed the occurrence of compounds including sesquiterpenes, megastigmanes, quinic acid derivatives, flavonoids, lignans, triterpenoid saponins, and polar lipids. The structures of these compounds were determined using 1D- and 2D-NMR analysis. In par-

ticular from the leaves of “Bianco di Pertosa” cv. an unreported sesquiterpene lactone belonging to the class of guaianolides was isolated.

Quantitative determination of caffeoyl- and dicaffeoyl-quinic acid derivatives was done using LC-ESI/QTrap/MS/MS, revealing 5-caffeoylquinic acid (chlorogenic acid) as the most abundant compound in both extracts. Furthermore, ¹H NMR spectra of the extracts revealed the presence of organic acids, sugars, and amino acids. The relative concentrations of the metabolites identified by ¹H NMR were determined by comparing them to the internal standard (TSP) using Chenomx software, which enables the quantification of individual components in NMR spectra.

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P233 Research and development on secondary metabolites of *Metarhizium anisopliae* under non-thermal plasma stimulation

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The entomopathogenic fungus *Metarhizium anisopliae sensu lato* is a pathogenic microorganism that infects insects and has been developed as an essential biopesticide. Therefore, the investigation on the secondary metabolites of *M. anisopliae* and effects to human health became an important issue. The technology in non-thermal plasma was applied on the study of its components and related functions [1, 2].

The effects of plasma stimulation and plasma-activated water (PAW) treatment on the changes of metabolite compositions during solid-state fermentation were explored in this study. High-performance liquid chromatography (HPLC) was used for compound identification and quantitative analysis. After stimulations using the methods in different conditions, the results in changes are insignificant.

The fungal mycelial and culture filtrate layers of *M. anisopliae* yielded by liquid fermentation were extracted, fractionated, separated, and purified, then eight pure compounds were isolated. The isolated compounds were structurally characterized by nuclear magnetic resonance (NMR) and electrospray ionization mass spectrometry (ESI-MS), etc. Eight compounds were identified as 5-hydroxymethyl-furan-2-carboxylic acid (1), hydroxybenzoic acid (2), 4-methoxyphenylacetic acid (3), ergosterol (4), helvolic acid (5), destruxin A (6), destruxin B (7), and destruxin E chlorohydrin (8). Among them, compounds 3 and 4 were reported to inhibit cyclooxygenase-2 (COX-2) activity and potentially affect platelet aggregation in previous studies. The progress of pharmacological test is on going.

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P234 Application of bioactivity-based molecular networking for the discovery of anti-inflammatory constituents from *Scaevola taccada* leaves

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Natural products have historically served as invaluable resources for drug discovery, despite the often time-consuming development process. Recent advances combining tandem mass spectrometry (MS/MS)-based molecular networking (MN) with biological activity assessment have significantly accelerated the identification of bioactive compounds. In this study, we employed bioactivity-based molecular networking (BBMN) to identify anti-inflammatory constituents from the leaves of *Scaevola taccada* (Gaertn.) Roxb. *S. taccada*, a native Taiwanese plant widely distributed across sandy and rocky coastal regions, possesses considerable economic value and has traditional medicinal applications. We evaluated the anti-inflammatory potential of extracts using an fMLP/CB-stimulated human neutrophil model, measuring inhibitory effects on both superoxide anion generation and elastase release. The BBMN analysis revealed bioactive clusters primarily comprising shikimates, phenylpropanoids, and terpenoids, which were prioritized for purification based on their anti-inflammatory properties. Through bioassay-guided fractionation of the active ethyl acetate-soluble fraction from *S. taccada* leaves, 15 compounds have been isolated and elucidated by spectral analysis. Among these, velutin demonstrated particularly potent anti-inflammatory activity. Our findings suggest that secondary metabolites from *S. taccada* leaves hold promising potential as therapeutic agents for inflammation-related disorders.

P235 Exploring anti-lipid droplet accumulation components from *Anredera cordifolia* using bioactivity-coupled MS/MS molecular networking

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The integration of tandem mass spectrometry (MS/MS)-based molecular networking (MN) with bioactivity-guided screening offers an efficient approach for isolating active compounds, substantially streamlining the purification process. Using a high-throughput screening platform, we evaluated the Taiwan edible plant for food extracts bank for anti-lipid droplet (LD) accumulation activity. The ethanolic extract of *Anredera cordifolia* (Ten.) Steenis leaves demonstrated remarkable inhibitory effects, suggesting its considerable potential as a reservoir of therapeutic compounds. We implemented a bioactivity-coupled MS/MS molecular networking strategy to identify potential anti-LD accumulation agents from this edible plant. This approach yielded one new compound (**AC-3**) and 26 known compounds spanning diverse chemical classes: one aliphatic (**AC-4**), one alkaloid (**AC-5**), four apocarotenoids (**AC-69**), 11 benzenoids (**AC-1021**), three chlorophylls (**AC-1**, **2**, and **22**), one coumarin (**AC-23**), one diterpenoid (**AC-24**), and three steroids (**AC-2527**). Notably, our bioactivity assessment revealed that two chlorophyll compounds (**AC-1** and **2**) exhibited pronounced inhibition of LD accumulation. These findings complement our earlier research, wherein methyl pheophorbide a — a chlorophyll derivative isolated from *Elaeagnus glabra* Thunb.—demonstrated significant anti-LD activity, further validating the therapeutic potential of chlorophyll-derived com-

pounds. Collectively, our findings highlight the rich phytochemical diversity and biological significance of *A. cordifolia*, establishing a robust foundation for its development as a natural therapeutic intervention for metabolic-associated fatty liver disease [1].

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P236 Is *Himantoglossum robertianum* a medicinal orchid? Unveiling a striking phytochemical parallel with *Gastrodia elata*

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Himantoglossum robertianum (Loisel.) P. Delforge is a widespread orchid of the Mediterranean region, where its hypogaeum organs are traditionally consumed as food [1]. Despite their ecological, medicinal, and evolutionary significance, orchids remain understudied in terms of their metabolite profile. For instance, aside from the volatile composition of flowers [2] and the identification of two dihydrophenanthrenes in the bulbs [3], nothing was known about the metabolome of *H. robertianum*. In this study, we provide the first metabolic fingerprinting of this orchid, identifying its signature compounds in bulbs, inflorescences, and leaves by NMR profiling (following the classical metabolomics protocol [4]) complemented by LC-MS.

Unexpectedly, our results revealed notable similarities between *H. robertianum* and *Gastrodia elata* Blume, a well-known orchid whose rhizome is used in Traditional Chinese Medicine for neurological disorders. All *H. robertianum* organs, especially the leaves, were found to be rich in gastrodigenin and its related compounds gastrodin, bis(4-hydroxy benzyl) ether, and parishins (parishin A, C, and E). Parishin A was the most abundant specialized metabolite, ranging from 43 to 104 mg/g DW in bulb and leaf, respectively. This suite of bioactive molecules has recognized pharmacological activities, particularly for the central nervous system [5]. Our findings suggest that *H. robertianum*, which has been cited in several ethnobotanical reports of the Mediterranean area, could represent a promising natural source of neuroactive compounds. Moreover, since leaves were identified as the richest source of these compounds, a more sustainable alternative to harvesting hypogaeum organs, as practiced with *G. elata*, can be hypothesized.

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P237 2-epi-Jaspine B inhibits breast cancer growth by inducing autophagy-mediated GRP78 in the cell surface

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Breast cancer is one of the common cancers and has a high mortality rate [1]. Therefore, discovering highly effective drugs with minimal side effects for breast cancer treatment is extremely important. 2-epi-Jaspine B (2-epi-JB) is the isomer of the active natural product Jasmine B isolated from the marine sponges *Jaspis* sp. [2]. 2-epi-JB has been reported to be cytotoxic to melanoma and hepatoma cells [2], but it has not been studied in breast cancer. In this study, we investigated the effect and mechanism of action of 2-epi-JB on breast cancer. 2-epi-JB suppressed the growth of breast cancer cells by inducing apoptosis and endoplasmic reticulum (ER) stress. Cell surface GRP78 (cs-GRP78) can trigger both pro-apoptotic and anti-apoptotic signaling pathways in cancer cells, making its role in cancer regulation controversial. Here, we confirmed that 2-epi-JB increased csGRP78. In addition, 2-epi-JB induced autophagy, which is important for maintaining homeostasis through degradation pathways [3]. 2-epi-JB activates autophagy by increasing p-AMPK α , Beclin1, ATG7, LC3, and decreasing p-mTOR. In addition, when autophagy was inhibited by 3-methyladenine, ER stress, csGRP78 expression, and cell growth were also inhibited. These findings suggest that 2-epi-JB-induced autophagy may be crucial for ER stress-csGRP78 accumulation-dependent cell death and that 2-epi-JB may be useful in the treatment of breast cancer.

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P238 Phytochemical profiling and cytotoxic potential of *Azadirachta indica* extracts against tumor cell lines

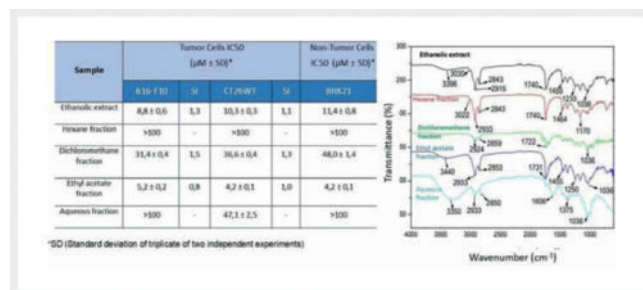
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Azadirachta indica (Neem) is a plant widely used in traditional medicine and currently studied for its pharmacological potential, particularly in oncology. This study aimed to extract, fractionate, and characterize bioactive compounds from Neem leaves and evaluate their cytotoxic activity against tumor cell lines (B16-F10 and CT26-WT) and non-tumor cell lines (BHK21). The extraction was conducted through ethanolic maceration (48 h), followed by fractionation using hexane, dichloromethane, ethyl acetate, and water [1]. The fractions were analyzed by Fourier Transform Infrared Spectroscopy (FTIR) and subjected to cell viability assays. The ethanolic extract and the ethyl acetate fraction exhibited the highest cytotoxic activity across all tested cells, whereas the hexane fraction did not show significant toxicity [2]. The aqueous fraction demonstrat-

ed selective action only against CT26-WT. FTIR analysis revealed that the ethanolic extract and ethyl acetate fraction displayed bands at 2933 cm⁻¹ and between 1450–1465 cm⁻¹, indicative of aromatic compounds such as flavonoids, tannins, and phenolics, which are associated with the observed biological activity. Signals at 2915 and 2873 cm⁻¹ suggested lipophilic chains, while bands between 1731–1740 cm⁻¹ indicated carbonyls related to esters and terpenes (► Fig. 1). In contrast, the hexane and dichloromethane fractions showed very weak signals between 3440–3390 cm⁻¹, indicating a low concentration of hydroxyl groups and, consequently, lower biological activity. The synergistic interaction among these compound classes may explain the pronounced cytotoxicity. Thus, the ethyl acetate fraction concentrates metabolites with antitumor potential, highlighting Neem as a promising source of bioactive compounds for pharmacological applications.



► Fig. 1 Cytotoxicity data and chemical characterization of the ethanolic extract and fractions of *Azadirachta indica* by Infrared Spectroscopy (FTIR).

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P239 A targeted mass spectrometric approach to evaluate the anti-inflammatory activity of the major metabolites of *Foeniculum vulgare* Mill. waste in human bronchial epithelium

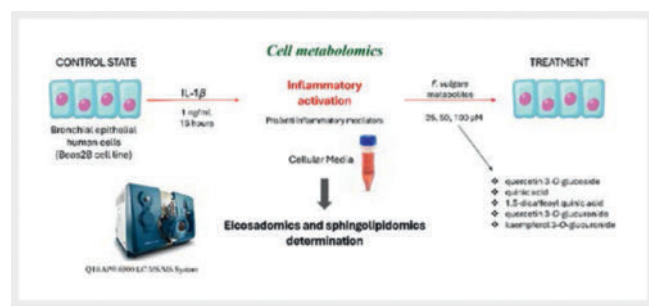
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Cell metabolomics offers deep insights into the mechanisms through which plant-derived bioactive compounds contribute to the prevention and mitigation of various diseases by modulating key metabolic pathways. The respiratory system is highly vulnerable to inflammation, with acute viral infections posing a significant global health risk. While many are mild and self-resolving, some can become severe. Lower respiratory tract infections—like bronchiolitis, pneumonia, and asthma or COPD exacerbations—can lead to respiratory failure,

serious complications, death, and lasting cardiovascular or neurological effects [1]. Given the anti-inflammatory properties of plant secondary metabolites, this study aimed at investigating the capacity of selected compounds extracted from *Foeniculum vulgare* waste to modulate the eicosanoid and sphingolipid inflammatory mediator pathways, in the respiratory tract [2]. Human bronchial epithelial cells (BEAS-2B cell line) were treated with quercetin-3-O-glucoside, quercetin-3-O-glucuronide, kaempferol-3-O-glucuronide, 1,5-dicaffeoylquinic acid, and quinic acid, isolated from fennel by-products. Inflammation was induced by IL-1 β stimulation, after which the cell culture supernatants were collected for metabolite extraction and purification. Eicosanoids and sphingolipids were subsequently quantified using UPLC-ESI-QTRAP-MS/MS in multiple reaction monitoring (MRM) mode. This study presents an innovative metabolomics-based approach to elucidate the mechanisms of action of natural compounds in cellular models. The findings reveal that fennel waste, rich in polyphenolic compounds, can attenuate inflammation in human bronchial epithelial cells by enhancing the production of anti-inflammatory mediators while suppressing pro-inflammatory pathways. These results suggest that functional foods or nutraceuticals derived from fennel waste can be used as promising adjuvants in the control of chronic inflammatory respiratory diseases, including COPD and asthma (► Fig. 1).



► Fig. 1 Research workflow.

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P240 Antibacterial and anti-inflammatory effects of wintergreen (*Gaultheria fragrantissima* Wall.) essential oil

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The essential oil of *Gaultheria* (GEO) species is known to contain methyl salicylate. This phenolic compound has high antimicrobial, insecticidal, and antioxidant effects [1], however, the biofilm inhibitory and antipsoriasis effects of GEO have not been investigated. The aim of our study was to determine the

chemical composition of *G. fragrantissima* oil (GFEO), to confirm its antibacterial activity and to investigate its anti-inflammatory effect.

GC-MS was used to determine the chemical constituents of GFEO. *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, methicillin-resistant *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 were included in the microbiological assays. Minimum inhibitory concentration (MIC) values were determined by microdilution method. Subsequently, biofilm formation inhibition studies were performed. An in vivo psoriasis model was used to test the anti-inflammatory effect.

The main component of GFEO was methyl salicylate. The microbiological tests showed that all pathogens were sensitive to GFEO, the most sensitive was *E. coli* (MIC: 0.156 mg/mL), the most resistant was *P. aeruginosa* (MIC: 2.5 mg/mL). GFEO had biofilm degrading activity on all bacteria, with the highest activity detected on *K. pneumoniae* (inhibition rate: 91.5%). In the psoriasis model, imiquimod treatment led to increased skin thickness, perfusion, and water loss. GFEO slightly reduced skin thickness compared to the positive control group.

Our results show that GFEO has both antibacterial and anti-inflammatory effects in vitro and in vivo. GFEO may be regarded as promising oil in complementary therapies.

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P241 Sweet cherry (*Prunus avium* L.) vegetal parts: Valuable source of bioactive compounds for therapeutic applications

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Sweet cherry is a highly valued fruit for its nutritional and biological properties. In Portugal, particularly in Fundão region, cherry processing generates about 15-30 % by-products annually. While some are used in traditional medicine, their bioactive potential remains underexplored. This study evaluated the phenolic, volatile, and mineral compositions of stems, leaves and flowers of the *Saco*⁻¹, with no cytotoxicity observed in NHDF cells. Anti-inflammatory potential was assessed in LPS-stimulated RAW 264.7 macrophages, where stems and leaves extracts inhibited nitrite production in a dose-dependent manner without cytotoxic effects. These findings provide valuable insight into the phytochemical composition and biological properties of Portuguese cherry by-products. Their bioactive potential suggests applications in health-related fields while also contributing to the valorization of local agricultural waste and the economy [1–3].

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P242 Exploring the Microbial Ecology of Sweet Cherry Fruits from Portugal

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Antibiotic effectiveness has been declining in recent years, owing mostly to the development of drug-resistant bacteria. Furthermore, because the majority of fruits and vegetables are consumed fresh, it is critical to see them as vectors for the spread of harmful microbes linked to illness outbreaks. As a result, there is a growing global interest in the microbial community of fruits and vegetables. As a result, understanding the food microbiome is crucial for food safety, consumption, growth control, and discovering novel sources of bioactive chemicals and beneficial bacteria. Among plants, *Prunus avium* fruits, i.e., sweet cherry fruits, have been a focus of several studies, chiefly due to their medicinal and health-promoting properties. Given the mentioned and the absence of data about sweet cherries microbiology, the present study aimed to assess the culturome of Portuguese sweet cherries over almost 3 months. For such, there were cultivated homogenates of sweet cherries on different broths. A total of 125×10^3 UFC/g bacteria and 212×10^2 UFC/g fungi were found on Trypticase soy agar and Sabouraud-Cloramphenicol media, respectively. The selected isolates were then molecularly identified by amplifying the 16S rRNA gene and ITS region. A total of 22 different bacteria and 33 fungi species were identified. *Pseudomonas* and *Ralstonia* were the most prevalent bacteria, while *Metchnikowia*, *Aureobasidium*, and *Hanseniaspora* were the most abundant fungi. The obtained data revealed that fruits ripen showed higher microbial diversity. The present investigation opens doors for further studies to reveal interactions between microbial species and the full safety of these fruits.

P243 Impact of controlled light stress on intracellular and extracellular polysaccharide accumulation in *Chlorella vulgaris* cultivated in an system indoor of integrated photobioreactors

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Chlorella vulgaris, a highly efficient unicellular photosynthetic organism, has gained attention as a sustainable source of biomass and bioactive compounds [1] due to its exceptional ability to utilize light and carbon dioxide, making it

valuable for industrial and environmental applications. Recent research highlights the advantages of cultivating microalgae in closed indoor photobioreactors (PBRs), which allow precise control of light, temperature, and nutrients while minimizing water loss [2]. This controlled environment improves photosynthetic efficiency and allows deliberate stress induction to influence metabolite production. As a result, the decreasing cost of microalgal biomass is enabling cost-effective exploitation of valuable metabolites such as polysaccharides [3]. In this study, an system indoor of integrated photobioreactors designed to maximize microalgal production was used to cultivate *C. vulgaris* SAG 211-12 in modified BG-11 medium. This system comprised an acceleration module (three column PBRs of 8, 16, and 32 L) and a production module (three 150 L annular PBRs). Cultures were maintained for 14 days under three light intensities, and growth was monitored via optical density (OD), cell counts, and dry weight. Medium light produced the highest biomass, whereas high light intensity induced stress that increased extracellular polysaccharide (EPS) secretion but reduced intracellular polysaccharide (CHL-A) content. CHL-A was extracted with hot water, and EPS was isolated through filtration and dialysis. Chemical and morphological analyses revealed structural and compositional differences between CHL-A and EPS. Overall, both CHL-A and EPS derived from *Chlorella vulgaris* exhibit promising potential as natural ingredients for sustainable industrial applications of microalgal products, given that indoor cultivation enables year-round, uninterrupted production regardless of external environmental conditions, a significant advantage over higher plants, which typically yield bioactive compounds only during specific seasonal (balsamic) periods.

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P244 Scientific evaluation to identify the inhibition of cytokine storm by Dasamoola Astadsanga Kwath: *In vitro* and *in silico* studies

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Dasamoola Astadsanga Kwath (DAK) is a polyherbal Ayurvedic formulation which is prescribed to patients suffering from viral infections. This formulation is useful in reducing the virulence of the virus which can be expressed in the

form of reduction in the chances of cytokine storm. The aim of the study is to evaluate the inhibition of cytokine storm by performing *in vitro* anti-inflammatory activity of DAK formulation and to predict *in silico* anti-viral activity of identified potential phytoconstituents. The MTT cell cytotoxicity assay, nitric oxide (NO) inhibition assay and pro-inflammatory cytokines assay were carried out at concentrations 100 and 200 µg/mL. The phytoconstituents were identified by UPLC-PDA and UPLC-HRMS analyses. For Ayurinformatics study, molecular docking and molecular dynamics analyses were used. The study revealed that DAK significantly inhibited NO release in comparison to dexamethasone (100 µg/mL) and pro-inflammatory cytokines in RAW264.7 cells. The two fractions, ethyl acetate and *n*-butanol prepared from the DAK formulation were non-cytotoxic against RAW264.7 murine macrophage cells during MTT cytotoxicity assay and showed satisfactory results during cytokines assay. *n*-butanol fraction contains active phytoconstituents in appreciable quantities. Twenty-two phytoconstituents have been identified by UPLC-HRMS analysis in the formulation and three phytocompounds were quantified by UPLC-PDA. In the present study, anti-inflammatory activity of DAK was evaluated and the claimed anti-viral property was re-confirmed by molecular modelling. These results indicate that the drug action of DAK against inhibition of cytokine storm; important phytoconstituents responsible for this drug action are epoxyaurapten, tribulusterine, myricetin, and pinoresinol.

P245 Effects of brassinosteroid on the accumulation of secondary metabolites in callus of *Aristolelia chilensis* (Mol.) Stuntz

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Aristolelia chilensis (Mol.) Stuntz, also known as Maqui, is an endemic plant native to southern Chile. It is characterised by its high value of bioactive compounds, mainly anthocyanins, indole alkaloids and flavonoids, coumarins, and delphinidin

3-O-β-glucoside [1]. These secondary metabolites (SM) are valued for their pharmaceutical, food and commercial applications. Brassinosteroids (BRs) are growth regulators [2] that could act as a potent natural elicitor influencing the biosynthesis of MS of interest *in vitro* culture [3]. Therefore, the effect of the addition of a BRs (BL) on maqui callus *in vitro* culture was evaluated with the aim of improving MS accumulation for potential antifungal use. Maqui callus cultures were grown on Gamborg B5 medium supplemented with different growth regulators (ANA (2 mg/L), GIB (0.5 mg/L), BAP (0.1 g/L)) for four weeks, then exposed for two weeks to BRs (BL; 0.04 mg/L). Extraction of maqui callus was carried out for the identification of anthocyanins, polyphenols and flavonoids by high performance liquid chromatography (HPLC-MS). The antifungal activity against *Monilinia fructicola* was determined by effective concentration (EC₅₀). According to HPLC-MS analysis, the callus culture treated exogenously with BRs accumulated higher concentrations of anthocyanin, flavonoids and phenolic compounds than untreated ones, which contributed to its efficacy as an antifungal agent. These results suggest that elicitation with BRs is an effective method to enhance the production of bioactive compounds in maqui.

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P246 Hyperuricemia exacerbates MRSA infection but is reversed by curcumin-based photodynamic therapy

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Hyperuricemia is associated with increased risk of infection-related mortality due to its capacity to impair immune responses. This condition is commonly observed in patients with severe bacterial infections, including those caused by methicillin-resistant *Staphylococcus aureus* (MRSA). Antimicrobial photodynamic therapy (aPDT) has emerged as a promising alternative to conventional antibiotics, using photosensitizers and light to generate reactive oxygen species that eliminate pathogens and modulate inflammation. Although curcumin-mediated aPDT has demonstrated efficacy against MRSA, its effects in hyperuricemic hosts remain unclear. This study evaluated the therapeutic impact of curcumin-based aPDT in MRSA-infected hyperuricemic BALB/c mice. Hyperuricemia was induced by intraperitoneal potassium oxonate (300 mg/kg) starting three days before infection and maintained throughout the experiment. Mice were intradermally infected with MRSA 43300 (1.5 × 10⁸ CFU) in the left ear and, after 24 hours, treated with curcumin (100 µg) followed by LED irradiation (450 nm, 13.5 J/cm², 3 min). Additional controls included: infected hyperuricemic mice (untreated, LED-only and curcumin-only) and normouricemic mice (infected and noninfected). After 24 hours post-aPDT, samples were collected for analysis. Ear lesion morphology, histology, and immunohistochemistry (MPO and anti-DNP) were assessed, along with plasma uric acid, and bacterial load, weight, and cytokine levels in lymph nodes. Potassium oxonate increased plasma uric acid levels by fivefold and exacerbated MRSA-induced inflammation, lesion size, leukocyte numbers, oxidative stress, and bacterial burden. Remarkably, curcumin-mediated aPDT reduced all parameters, significantly ameliorating all pathological markers. These findings support curcumin-associated aPDT as a promising strategy for controlling MRSA skin infections, particularly in hyperuricemic or inflammation-prone conditions [1–5].

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P247 Selective bioactivity in *Melipona mondury* geopoly: seasonal influence on photodynamic, antibacterial, and antiproliferative effects

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Melipona mondury, a stingless bee, produces geopoly, a resinous product with promising pharmacological potential whose composition and bioactivity may vary with seasonal factors. This study evaluated the influence of seasonality on the chemical profile and biological activities of geopoly samples collected in May, June, July, October, November, and December 2023. Ethanol extracts and their hexane, ethyl acetate, and butanolic fractions were analyzed for total phenolic content, antioxidant activity (DPPH• and ABTS•⁺ assays), antibacterial action (against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and methicillin-resistant *S. aureus* [MRSA] under blue LED light), and antiproliferative effects (on HepG2 and HCT116 cell lines). The June butanolic fraction exhibited the highest phenolic content, antioxidant activity, and bactericidal effect against *P. aeruginosa* (MIC = MBC = 100 µg/mL), as well as light-independent bactericidal activity against MRSA (IC₅₀ = 7 µg/mL). In contrast, the June ethyl acetate and hexane fractions killed MRSA only under LED exposure (IC₅₀ = 0.43 and 0.30 µg/mL, respectively). The ethyl acetate fraction also inhibited HepG2 and HCT116 proliferation (IC₅₀ = 17.8 and 18.9 µg/mL). ESI-MS/MS (negative mode) of this fraction revealed ions corresponding to cinnamic, salicylic, gallic, and trimethyl acids. The butanolic and hexane fractions presented a predominant *m/z* 169 ion (gallic acid), and a previously unreported *m/z* 527 ion, potentially linked to environmental specificity. Palynological analysis identified pollen from *Eucalyptus*, *Schinus terebinthifolius*, and Euphorbiaceae in all samples. These findings highlight the strong influence of seasonal variation on the bioactive potential of geopoly and, notably, report for the first time its photosensitizing effect against MRSA, revealing new prospects for light-activated antibacterial agents [1–4].

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P248 Deep-sea *Streptomyces* spp. as a source of bioactive secondary metabolites and new siderophores

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The phylum Actinomycetota is predominantly responsible for producing the majority of marine microbial secondary metabolites of which the genus *Streptomyces* is the most prolific, biosynthesizing most of the well-known clinically useful antibiotics and other bioactive compounds, including siderophores that scavenge iron from the environment. Some well-known *Streptomyces*-derived siderophores, studied within the EU's Horizon2020 SECRET project, have biomedical and environmental applications. In continuation of our previous studies on the genus *Streptomyces* [1–2], in the present study, emphasis was given on the discovery of novel siderophores. Novel approaches such as computational mass spectrometry and molecular networking (MN) were applied with the purpose of exploring the chemical profile of the extracts and identifying related analogues. Liquid culture supernatants of three *Streptomyces* strains were selected due to their siderophore content (*S. coelicoflavus*, *S. tri-tolerans* and *S. sclerotialis*) and screened for their secondary metabolites. The crude extracts were subjected to chromatography techniques for the separation of the molecules. Standard TLC was conducted for an initial screening using different reagents, including FeCl₃ for siderophore detection. For the structural elucidation of the isolated compounds, 1- and 2-D Nuclear Magnetic Resonance (NMR) spectroscopy and Mass Spectrometry (MS) were employed. In order to obtain pure compounds, High Performance Liquid Chromatography (HPLC) and Preparative Thin Layer Chromatography (PTLC) were used. Overall, fifteen compounds were isolated and identified: one cyclic hydroxamic acid siderophore, two diketopiperazines, five a-pyranones, one tyramine alkaloid, three benzoic acid derivatives, one benzodioxole derivative, one purine nucleoside and one novel madurastatin analogue.

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P249 Bioactive natural prodiginine/prodigiosin pigments from the deep-sea-derived actinobacterium *Streptomyces coelicoflavus*

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The phylum *Actinomycetota* represents one of the most extensive taxonomic groups within the domain *Bacteria*, with *Streptomyces* being one of its most prominent genera regarding its biosynthetic potential and biotechnological applications. An interesting group of bioactive compounds derived from various bacterial phyla, including *Actinomycetota*, are prodiginines, including prodigiosin—red-pigmented molecules with anticancer and antibacterial activities [1]. While initial studies hint at its bioactive promise [2], the species under study, *Streptomyces coelicoflavus*, remains chemically underexplored, especially regarding its pigment production. Herein, we present a workflow for the successful detection, isolation and structural elucidation of bioactive natural pigments of the prodiginine/ prodigiosin family from the intensely colored biomass of the marine actinobacterium *S. coelicoflavus*. The extracts were acquired through a multi-step ultrasound-assisted extraction of the biomass originating from liquid cultures, and then subjected to MPLC fractionation. Identification of the compounds present in the crude extract was carried out by a GC–MS analysis. Two major pigmented compounds were assigned by comparing their mass spectra to those found in reference libraries and were additionally structurally elucidated via NMR as undecylprodigiosin (= undecylprodiginine) and metacycloprodigiosin (= metacycloprodiginine) (► Fig 1).



► Fig. 1 The structures of bioactive pigments isolated from *Streptomyces coelicoflavus*.

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P250 Identification of catechin derivatives in the flowers of *Camellia japonica*

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Catechin derivatives are compounds belonging to the flavanol family. They possess activities described in the literature that may be of interest for personal care products, such as anti-inflammatory or antioxidant properties [1]. While they are fairly well described in *Camellia sinensis*, this class of compounds is much less studied in other species of the genus *Camellia*. In particular, few documents mention the presence of procyanidins in *Camellia japonica*, but to our knowledge, none offers a validated identification at the molecular level. In this study, chemical identification has been undertaken on procyanidins, condensed tannins of catechin and epicatechin, present in the flowers of *Camellia japonica*. Analyses were conducted using various analytical methods such as liquid chromatography (LC) coupled with diode array detector and evaporative light scattering detector (DAD–ELSD), or Orbitrap high resolution mass spectrometry (HRMS), as well as flash chromatography and preparative chromatography.

Finally, in addition to catechin and epicatechin, 5 procyanidins were identified at level 1 (validated identification according to classification used by the Center

for Innovative Technology) and 13 at level 2 (putative identification), including 12 compounds cited for the first time in *Camellia japonica* flowers.

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P251 Bio-guided isolation of tyrosinase inhibitors produced by *Amycolatopsis* strain derived from the Greek ecosystem

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Actinobacteria from Greek ecosystems are renowned for their ability to produce bioactive compounds, yet their rich chemodiversity remains largely unexplored [1]. As part of our screening project for the discovery of molecules of microbial origin with skin-whitening activity, 1000 isolates originating from the Greek natural environment and belonging to the ATHUBA collection (Athens University Bacterial & Archaea Culture Collection) were investigated. The microbial strain Q6 was selected as the most promising, while phylogenetic analysis revealed that it could represent a new species within the genus *Amycolatopsis*. The effect of different fermentation conditions on the production of tyrosinase inhibitory activity was examined, in order to identify the optimum cultivation conditions. LCMS based metabolomics was applied to determine significant differences between the fermentation conditions, and to identify potential bioactive secondary metabolites. A customized bio-guided workflow was then applied, which combined a high-throughput dereplication and molecular networking method (UHPLC–HRMS), along with tyrosinase-based HPTLC–bioautography and the bioactivity of each fraction. Bioassay-guided purification of the main active components led to the isolation and structure elucidation of key compounds using 1&2D NMR. The ultimate goal of this study is the targeted isolation and identification of bioactive compounds, which can be integrated into cosmeceutical formulations. The latter findings highlight the significant potential for exploiting the Hellenic microbial diversity in the realm of anti-aging research.

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P252 Exploring Brazilian biodiversity: a study of phytochemical diversity in the genus *Cololobus*

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The Atlantic Forest is one of the most biodiverse ecosystems in the world, playing a pivotal role in the conservation of plant species [1]. The genus *Cololobus* is native to the Southeastern region of Brazil, presenting species endemic to the Atlantic

Forest. Currently, the genus is composed by five described species, namely: *Cololobus argenteus*, *Cololobus hatschbachii*, *Cololobus longiangustatus*, *Cololobus rupestris* and *Cololobus ruschianus* 2–4]. Herein, we employed liquid chromatography-tandem mass spectrometry-based metabolomics approach to evaluate the chemical diversity, measured as metabolite richness and evenness, of five *Cololobus* species, including three known species (*C. argenteus*, *C. longiangustatus* and *C. ruschianus*) and two potentially novel species. By investigating these species, our goal was to assess whether novel species share chemical similarities with previously described ones, broadening the knowledge of Brazilian biodiversity. Regarding the known species, we observed the highest metabolite richness in *C. longiangustatus* (126 metabolites), followed by *C. ruschianus* (71 metabolites) and *C. argenteus* (34 metabolites). A similar profile was observed for metabolite evenness, measured as Simpson's index, where *C. longiangustatus* exhibited greater metabolite diversity (0.9625) than *C. ruschianus* (0.9318) or *C. argenteus* (0.9195). Interestingly, novel species exhibited metabolite richness and evenness comparable to the measurements obtained for *C. longiangustatus* and *C. ruschianus*. Additionally, principal component analysis corroborated the results observed for phytochemical diversity, where the two novel species were chemically similar to *C. longiangustatus* and *C. ruschianus*. Our results shed light on the phytochemical diversity of endemic plant species in the Atlantic Forest, enriching the understanding of Brazilian biodiversity.

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P253 New acylphloroglucinols from *Hypericum hircinum* L.

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In continuation to our previous studies on the genus *Hypericum*, we present here the first isolation of polycyclic polyprenylated acylphloroglucinols (PPAPs) from *H. hircinum* L. The entire fractionation process was continuously monitored using ¹H-NMR to identify the characteristic prenyl protons or low field shifted protons involved in H-bonds [1].

A petroleum ether extract was obtained from the dried aerial parts by percolation. Afterwards the extract was defatted by column chromatography using Diaion (eluted with 90 % MeOH, 100 % MeOH, dichloromethane and hexane). The obtained 100 % MeOH fraction was fractionated by flash chromatography on silica gel using hexane-ethyl acetate-methanol mixtures. Further purification on RP18-HPLC, yielded so far, one known PPAP previously described as hyper-scabrone A [2] and seven new derivatives with the same basic structure (type A PPAPs, Fig. 1). Structure elucidation was based on HRESIMS and ¹H-, ¹³C- and 2D NMR spectroscopy. The relative stereochemistry was determined by NOESY and comparison with literature data.

Interestingly, a broad structural variation of the isolated acylphloroglucinols was observed, however all of them possess a benzoyl group. Moreover, several derivatives contain a geranyl instead of an isoprenyl substituent compared to similar compounds. A few derivatives also possess a furano or pyrano ring, in different position of the phloroglucinol nucleus. Structure elucidation of further isolated compounds is still in progress.

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P254 *In vitro* enzymatic inhibitory activities of Echinoderm extracts

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Damaging free radicals instigate a chain of biochemical reactions that give rise to notable degenerative diseases such as diabetes mellitus, neurodegenerative diseases such as Alzheimer's disease, cancer, and various cardiovascular diseases, which are all included in the top ten causes of death worldwide in 2020 by the World Health Organization (WHO). [3]

The study aimed to evaluate the antidiabetic, anti-inflammatory, neuroprotective, and antihypertensive activities of the hexane (H) and methanol (M) crude extracts of five (5) Echinoderm species, namely, *Marthasterias glacialis* (Mg), *Strongylocentrotus droebachiensis* (Sd), *Acanthaster planci* (Ap), *Choriaster granulatus* (Cg), and *Culcita novaeguineae* (Cn). The crude extracts were subjected to α-glucosidase, 15-LOX, Acetylcholine Esterase (AChE), and Angiotensin Converting Enzyme (ACE) inhibitory assays [1, 2]. Results revealed that among the extracts, ApH exhibited the highest α-glucosidase inhibition (72.85 %) followed by SdM (50.65 %), and CgH (28.41 %) at a well concentration of 20 µg/mL. Anti-inflammatory activities of CnM, ApH, MgH were 28.24 %, 19.77 %, and 10.81 %, respectively at 10 µg/mL well concentration. Compared to the control, MgM inhibited AChE activity by 18.68 %, ApH by 16.89 %, and CgM by 14.48 % at 100 µg/mL. ACE inhibition was registered by almost all the extracts with ApM (55.71 %) leading, followed by CgH (53.43 %), and CgM (53.14 %) compared to the control at 16.67 µg/mL. Among the Echinoderm species studied, Ap and Cg show antidiabetic and antihypertensive potentials. The authors declare no conflict of interest.

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P255 Antidiabetic potential of *Ficus capensis*, *F. vogelii*, and *Terminalia mollis*: a bioguided study

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Ficus capensis, *F. vogelii* and *Terminalia mollis* grow wild in Sub-Saharan Africa and have been used in folkmedicine as hypoglycemic agents, thus the aim of this study is to assess their antidiabetic potential.

Fractionation of the methanolic leaf extracts was performed using open column chromatography with Diaion HP20 (eluted with 90 % MeOH, 100 % MeOH, di-

chloromethane and hexane), followed by Sephadex LH20 (eluted with MeOH). Bioactive fractions were identified using the α -amylase and α -glucosidase inhibition assays, as described before [1, 2]. TLC analysis indicated that the active fractions of *F. capensis* and *F. vogelii* contained condensed tannins, as shown by derivatization with 1% vanillin/HCl, while *T. mollis* contained hydrolysable tannins, detected using 1% FeCl₃. The IC₅₀ values for α -amylase are 186.7, 72.9 and 234.5 μ g/ml, and for α -glucosidase are 8.9, 2.9 and 0.5, for *F. capensis*, *F. vogelii* and *T. mollis*, respectively. Structural characterization of the polymeric tannins was performed using ¹³C NMR spectroscopy (deuterated acetone:water, 298 K, Avance III 600 HD). These findings support the traditional use of these plants as antidiabetic agents and highlight the potential of plant tannins as promising bioactive compounds for diabetes management.

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P256 Phytochemical profiling of *Lavandula officinalis* ethanolic extract: potential neuroactive metabolites for gut microbiota biotransformation

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Lavender (*Lavandula officinalis*) infusion has been clinically demonstrated to alleviate symptoms of mild depression and anxiety with efficacy comparable to that of selective serotonin reuptake inhibitors (SSRIs) [1, 2]. However, the underlying mechanisms responsible for these effects remain poorly understood. Current hypotheses based on in vitro assays of isolated phytochemicals offer limited and inconclusive insights [3]. Emerging evidence suggests that the gut microbiota may metabolize plant-derived compounds into bioactive metabolites capable of modulating neurophysiological processes via the gut-brain axis [4, 5]. To investigate this possibility, a crucial first step involves the isolation and structural characterization of the constituent compounds in lavender extract.

The aim of the present study was to isolate and characterize secondary metabolites from the ethanolic extract of *Lavandula officinalis* flowers. The extract was subjected to sequential solvent partitioning with increasing polarity, followed by flash chromatography, and final purification using preparative HPLC. The isolated compounds were structurally elucidated using mass spectrometry (HPLC-DADIT-MS/MS) and NMR spectroscopy.

The isolated compounds were classified into two major groups: phenolic acids and flavonoids. Phenolic acids included primarily umbellic, *o*-coumaric, and caffeic acid derivatives with frequent methoxylation. Second groups consist of derivatives of apigenin, luteolin, and tricetin. Phenolic acids and flavonoids were predominantly conjugated through ether linkages with glucose and glucuronic acid residues and formed complex structures. The most abundant compounds in extract were *E/Z*-melilotoside and *E/Z*-4-methoxy-melilotoside. These structurally diverse metabolites represent potential substrates for gut microbial metabolism. Future studies will focus on evaluating their biotransformation by gut microbiota and assessing their neuroactive potential.

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P257 Integrated biological and metabolomic profiling to identify novel Respiratory Syncytial VIRUS (RSV) antiviral natural products

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Respiratory syncytial virus (RSV) is a leading cause of bronchiolitis in children, with no effective treatment available [1]. Natural products, long recognized as a cornerstone of drug discovery, offer significant potential to address this unmet need. To identify new antiviral molecules, we screened 800 plant natural extracts (NEs) prepared from the Pierre-Fabre Laboratoires (PFL) [2] using a fluorescence cell-based assay. NEs exhibiting antiviral activity in RSV-infected cells while maintaining > 75% cell viability were further confirmed in an apical-out airway organoid model. Simultaneously, extracts were systematically characterized through integrated UHPLC-HRMS² metabolite profiling analysis.

We prioritized NEs with fewer reported compounds and excluded those containing known antivirals using metabolomics and annotation tools. This approach led to the identification of four promising NEs. The active compounds were isolated by high-resolution semi-prep. HPLC chromatography combining the gradient transfer and dry load injection [3]. Structure elucidation was performed by UV, HRMS, and NMR. EC₅₀ values of pure compounds were comparable to or exceeding those of the reference compound, ribavirin. The identified molecules encompass a diverse range of chemical classes, including chalcones, isoquinoline alkaloids, indole alkaloids, and stilbene derivatives.

Further experiments will elucidate their mechanism of action and the stage in RSV. Subsequently, the most promising molecules will be tested in ex vivo reconstituted human airway epithelial tissue to further assess their therapeutic potential in more physiological conditions. This study highlights the effectiveness of our screening workflow in identifying novel RSV inhibitors and advancing antiviral drug discovery.

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P258 Natural compounds as CYP450 inhibitor scaffolds: Restoring insecticide efficacy through plant-based synergists

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Mosquito-borne diseases remain a major health challenge [1]. Vector control relies heavily on synthetic insecticides [2], which have led to widespread insecticide resistance, largely due to the overexpression of cytochrome P450 monooxygenases (CYPs) [3]. To identify plant-based synergists capable of counteracting metabolic resistance, over 70 extracts and natural compounds from a curated in-house library were screened for inhibitory activity against mosquito and pest detoxification enzymes, including *Anopheles gambiae* CYP9K1 and *Bemisia tabaci* CYP6CM1, using a high-throughput *in vitro* fluorescent assay. The results revealed 14 moderate inhibitors of AgCYP9K1. The most promising hit underwent phytochemical analysis via UPLC-PDA and UH-PLC-HRMS/MS, which identified its major constituent. The purified compound exhibited notable inhibitory activity ($IC_{50} = 18.37 \mu M$), while a semisynthetic analogue—designed to enhance binding affinity through structural modification—demonstrated significantly improved potency ($IC_{50} = 2.50 \mu M$). *In vivo* assays using pyrethroid-resistant *An. gambiae* confirmed that neither compound exhibited intrinsic toxicity. However, both enhanced deltamethrin efficacy, with the semisynthetic analogue producing a more pronounced synergistic effect compared to the plant-derived compound. This study highlights the potential of natural products as CYP450 inhibitors and insecticide synergists. Our findings support the rational discovery of bioactive phytochemicals as biopesticide additives to restore insecticide efficacy and manage resistance in mosquito vector populations.

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P259 Ability of flavonoids to inhibit intestinal α -glucosidases

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Type 2 diabetes is one of the most prevalent diseases which is associated with impaired regulation of postprandial hyperglycemia. α -glucosidase inhibitors can slow the release of glucose from starch and oligosaccharides, delaying glucose absorption and reducing postprandial blood glucose levels [1]. Flavonoids, secondary plant metabolites commonly found in our diet, have demonstrated various health benefits. Several studies have also confirmed that they can inhibit α -glucosidase activity *in vitro*, improving postprandial glycaemia in diabetic animal models and, to a limited extent, in human studies [2, 3].

For this reason, twenty-six flavonoids and nine polyphenols were tested for their ability to block α -glucosidases. Both yeast and mammalian (rat) glucosidases were used. While isoflavonoids and coumarins showed a rather mild effect, flavonols including their glycosides were potent inhibitors. One of the most potent compounds was kaempferol, which inhibited α -glucosidase more effectively than the registered drug acarbose, which was chosen as the standard agent. There were significant discrepancies between yeast and mammalian (rat) intestinal α -glucosidase inhibition. Yeast α -glucosidase does not appear to be a suitable model to predict effects in humans.

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P260 *In silico* identification and *in vitro* evaluation of natural products targeting the bacterial cell wall synthesis enzyme Filamenting temperature-sensitive mutant Z (FtsZ)

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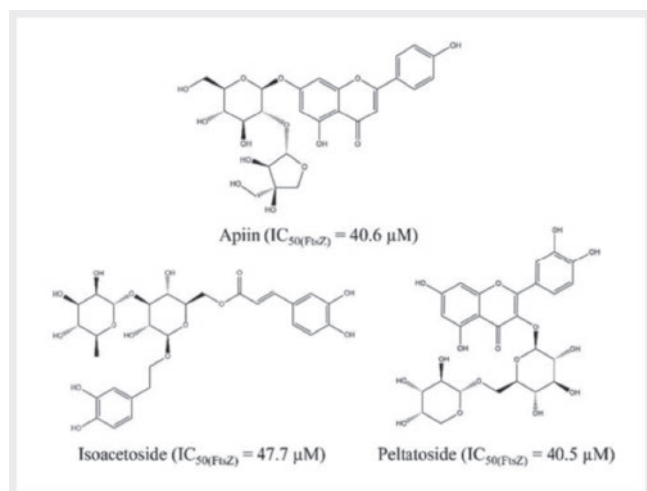
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Rising antimicrobial resistance is threatening the effectiveness of antibacterial therapies all over the world. Hence, the development of innovative anti-infective therapies is one major challenge of today's drug development research. Due to the specific physiological relevance of the bacterial cell wall, its biosynthesis is an important target for effective antibiotics. Nevertheless, for a variety of promising target proteins involved in the complex bacterial cell wall synthesis, no inhibitor for therapeutic purposes is available today.

To identify new leads against such an "unaddressed" target enzyme, a pharmacophore-based virtual screening was carried out on the cell wall synthesis protein *Filamenting temperature-sensitive mutant Z* (FtsZ) [1], employing a natural product (NP) database consisting of around 500 entries available for *in vitro* testing. A set of 12 *in silico*-identified NPs was subsequently evaluated against recombinant *Escherichia coli* FtsZ by a spectrophotometric inhibition assay and by monitoring FtsZ filamentation via Atomic Force Microscopy (AFM). In a final step, the impact of the *in silico* hits on whole *E. coli* cells and on murein-sacculus assembly was investigated by nanoscopic AFM imaging on isolated *E. coli* peptidoglycan sacculi in comparison to untreated controls.

The described techniques led to the identification of three previously unknown NP FtsZ inhibitors (► **Fig. 1**) with experimentally determined IC_{50} values around 40 μM . Compared to the commonly known natural product FtsZ inhibitor berberine ($IC_{50} = 51.37 \mu M$), the new inhibitors show similar inhibitory activity, possibly allowing their use as lead structures for rational FtsZ inhibitor design in the future.



► **Fig. 1** The three most active FtsZ inhibitors identified in this study and their corresponding IC_{50} (FtsZ) values

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P261 Discovery of structurally distinctive terpenoid derivatives from anti-inflammatory fractions of *Syzygium tripinnatum* leaves

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The *Syzygium* genus (Myrtaceae family) demonstrates diverse bioactivities with significant development potential [1]. Among its members, *Syzygium tripinnatum*, native to Taiwan, remains largely unexplored with only nine compounds previously isolated and minimal characterization of its bioactive properties. Preliminary research revealed that different fractions of *S. tripinnatum* exhibit distinct anti-inflammatory mechanisms: the *n*-hexane fraction promoted Nrf2 activation, the ethyl acetate fraction inhibited elastase release, and the water fraction suppressed IL-6 production. Phytochemical investigation of the *n*-hexane fraction yielded six new compounds: two terpenoid-conjugated phloroglucinols (syzygidiones A-B, 1-2), two novel triterpenoids (tripiszygin A, 3 and (20R)-taraxastane-3 β ,20 β -diol-28-al, 4), and two new sesquiterpenoids (syzygiterpenes A-B, 5-6), together with one triterpenoid first isolated from natural source ((3 β)-3,28-dihydroxyoleanan-12-one, 7). Additionally, 28 known compounds were identified, including a terpenoid-conjugated phloroglucinol (8), triterpenoids (9-26), α -tocopherol derivatives (27-29), chlorophylls (30-31), steroids (32-33), a benzenoid (34), and a sesquiterpenoid (35). Compounds available in sufficient quantities were evaluated for anti-inflammatory activity. Notably, syzygidiones A (1) and B (2) represent phloroglucinols conjugated with sesquiterpenes, featuring a structural scaffold previously reported only within the Myrtaceae family. This study marks the first report of terpenoid-conjugated phloroglucinols from the *Syzygium* genus, significantly expanding its known phytochemical profile. Furthermore, tripiszygin A (3) presents an unusual triterpenoid structure with a distinctive double bond at C-1/C-10 and an ether bridge connecting rings A and B. This comprehensive investigation of

bioactive compounds from *S. tripinnatum* provides new opportunities for the discovery and development of anti-inflammatory therapeutics.

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P262 A study on the triterpenoid constituents in *Daboecia cantabrigia* and their effects on cell proliferation, invasion and cellular migration

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Daboecia cantabrigia is primarily distributed across Europe, including Western Ireland, particularly in counties Galway and Mayo. *D. cantabrigia*, commonly referred to as Saint Dabeoc's Heath, belongs to the Ericaceae family [1]. This study aims to completely identify the terpenoid constituents in *D. cantabrigia*. The study focused on the identification of the triterpene constituents in the ethyl acetate extract of the leaves and flowers of the plant using gas chromatography-mass spectrometry (GC-MS). Furthermore, the potential anticancer activity of individualized terpenoid fractions were evaluated using cell proliferation, migration, and invasion assays using human umbilical vein endothelial cells (HUVECs) and prostate cancer cells (PC-3). The ethyl acetate extract of the leaves was prepared and subjected to flash column chromatography to collect individual fractionated bands. A gradient mobile phase system, employing varying ratios of hexane and ethyl acetate, was utilized for the separation process, and resulted in the isolation of five fractions. Constituents contained therein were identified and quantified using GCMS. These were found to be rich in lupeol, α -amyrin, ursolic acid, and oleanolic acid with additional minor compounds present including β -amyrone, α -amyrone, lupenone, erythrodiol, uvaol, betulin, ursolic aldehyde, and β -sitosterol. The triterpenoid fraction rich in ursolic acid demonstrated significant activity across all cell-based assays measuring the degree of proliferation, invasion and migratory effects of both HUVECs and PC-3 cells.

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P263 Bimatoprost induces the proliferation of NIH3T3 fibroblasts by activating Akt/ β -catenin via KATP channels

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Prostaglandins (PGs) regulate a variety of biological processes including inflammation, cell differentiation, and proliferation [1]. Bimatoprost, a structural analog of PGF $_{2\alpha}$, is used in the treatment of glaucoma and is recognized as the first FDA-approved agent for eyelash enhancement [2]. Despite its potential, there is limited information on the role of bimatoprost in fibroblasts, which are crucial for the organization of the extracellular matrix and tissues. Here, we investigated the effects and mechanisms of bimatoprost on the proliferation of NIH3T3 fibroblasts. Bimatoprost induced an increase in the proliferation of NIH3T3 fibroblasts by decreasing the number of subG1 cells and increasing the number of G1 cells. We observed that bimatoprost increased the levels of ac-

tivated Akt, β -catenin and its target gene, Cox-2. Interestingly, glibenclamide, an ATP-sensitive potassium (KATP) channel blocker, inhibited the activation of Akt and cell proliferation by bimatoprost. Moreover, bimatoprost-induced up-regulation of β -catenin and Cox-2 levels was attenuated by pretreatment with glibenclamide. These results suggest that bimatoprost induces proliferation of NIH3T3 fibroblast by activating the Akt/ β -catenin via the KATP channel, and that bimatoprost can be used for regeneration in damaged fibroblast-rich tissues.

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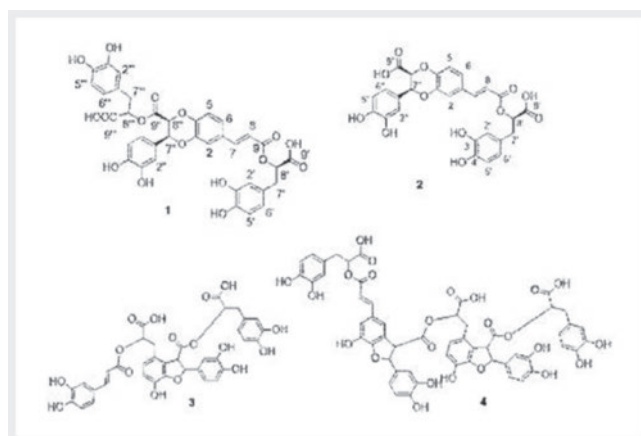
P264 Caffeic acid oligomers from *Origanum dictamnus* and *Satureja pilosa* as selective inhibitors of carbonic anhydrases: structure elucidation, X-ray crystallography

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In the present study four depsides were isolated from *Origanum dictamnus* L. and *Satureja pilosa* Velen [1, 2] and were fully characterized spectroscopically by means of 1D- and 2D- NMR, HRMS and Circular Dichroism analyses. Experimental ECD measurements and ECD calculations confirmed the absolute configuration of salvianolic acid P 2 to be (8'R,7''S,8''S) [1]. Despite the absolute configuration of the newly reported dittanic acid **1** could not be determined, due to its superior molecular complexity and flexibility, the consistency between ECD spectra of **1** and **2** suggested the stereogenic centers being identical in both compounds. All NPs **1-4** (► Fig. 1) inhibited in vitro the tumor associated isoforms hCA IX and XII in low micromolar concentration ranges and with high degree of selectivity over the constitutive hCAs I and II. X-ray crystallography on the hCA II/salvianolic acid P 2 adduct demonstrated that the ligand established a multitude of stabilizing interactions within the catalytic cleft. Specifically, the two catechol moieties were engaged in hydrogen bonds with the ZBW and the DW placed at the bottom of the cavity as well as with aminoacids defining the rim of the active site. Finally, compounds **1-4** were assayed for their cytotoxicity on a prototypic human triple negative breast cancer cell line MDA-MB-231 and exerted significant cytotoxic effects. Although the mechanisms of action of **1-4** are likely to ascribe to a multitude of targets, our results strongly suggest that this class of oligomeric depsides is indeed a promising group of phenolic compounds worth of further investigation.



► Fig. 1 Structures of compounds 1-4

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P265 Substrate-Driven metabolite diversification in *Hericium erinaceus*: Enhanced anti-*Helicobacter pylori* activity via brown rice cultivation

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This study aimed to develop high-quality *Hericium erinaceus* (lion's mane mushroom) by the regulation of cultivation conditions. Eco-friendly cultivation strategy for *H. erinaceus* was developed by substituting conventional sawdust with brown rice as a growth substrate. Metabolomic profiling revealed distinct chemical compositions depending on the substrate: hericenone A was identified as the principal compound in sawdust-grown fruiting bodies, whereas *N*-dephe-nylethyl isohericin (NDPIH) dominated in specimens cultivated on brown rice. Remarkably, extracts from brown rice-grown mushrooms exhibited significantly enhanced anti-*Helicobacter pylori* activity, attributed to the potent inhibitory effects of NDPIH, which surpassed the comparatively modest activity of hericenone A. Molecular networking-guided isolation led to the identification of nine additional compounds, including five isoindolinones and four isobenzofuranones, among which three new metabolites were characterized and designated as hericerinones A-C. Structure-activity relationship analysis suggested that variations in functional groups influenced anti-*H. pylori* efficacy. These results demonstrate that substrate composition modulates secondary metabolite biosynthesis and associated bioactivities in *H. erinaceus*. Brown rice not only induces a unique bioactive metabolite profile with therapeutic potential against gastrointestinal pathogens but also offers a sustainable, edible, and cost-effective alternative to conventional cultivation media [1].

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P266 Molecular networking-guided identification of bioactive compounds from *Phyla nodiflora* green extract protecting keratinocytes against PM-induced oxidative stress via the Nrf2/HO-1 pathway

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Ambient particulate matter (PM) is a major air pollutant associated with various skin disorders, including inflammation, atopic dermatitis, and psoriasis [1, 2]. Upon PM exposure, excessive reactive oxygen species (ROS) are generated in the skin, leading to oxidative stress, inflammation, and cellular dysfunction [3]. *Phyla nodiflora* (PN), a perennial herb in the Verbenaceae family, was selected for investigation due to its documented anti-inflammatory, anti-oxidant, and anti-microbial activities [4, 5]. These properties suggest it may offer protective effects against PM-induced skin damage. In this study, we aimed to evaluate the protective effects of PN against PM-induced oxidative damage in human keratinocytes (HaCaT cells). A solvent-free microwave hydrodiffusion and gravity (SF-MHG) extraction method was employed to obtain PN extracts and fractionate them into different polar components. Cell viability (resazurin assay) and intracellular ROS levels (H2DCFDA assay) were used to assess cytoprotective activity. Among the PN fractions, Frs. 4 and 5 significantly reduced ROS levels (> 50 %) in PM-treated HaCaT cells without cytotoxicity at concentrations below 30 µg/mL. Western blot analysis revealed that PN treatment upregulated Nrf2 and HO-1 expression, suggesting activation of the Nrf2/HO-1 antioxidant pathway. Furthermore, molecular networking based on LC-MS/MS data guided the identification of putative bioactive compounds responsible for the observed activity. Overall, this study highlights *Phyla nodiflora* as a promising natural resource for protecting the skin against PM-induced oxidative stress through green extraction and bioactivity-guided fractionation. Further investigation of its protective mechanisms is ongoing.

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P267 Re-evaluating natural compounds for antimicrobial use: essential oils and their activity against *Escherichia coli*

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Antibiotic resistance is becoming a significant global problem. Pharmaceutical companies are investing less in the development of new antibiotics, making basic research in this area increasingly important. Essential oils have emerged as promising natural alternatives due to their antimicrobial activity, including against multidrug-resistant bacteria, either alone or in synergy with antibiotics. The aim of our study was to investigate the antimicrobial activity of selected, lesser-explored essential oils against *Escherichia coli*, a clinically relevant pathogen [1–3].

We tested 20 commercially available essential oils, including *Thymus vulgaris* as a reference due to its well-established antimicrobial properties. Because essential oils do not diffuse well in hydrophilic media, the disc diffusion method proved unsuitable. Instead, we used a liquid growth medium to assess antibacterial activity. Chemical composition of the essential oils was determined using gas chromatography-mass spectrometry (GC-MS).

The essential oil of *Thymus vulgaris* contained 50 % thymol, 18 % *para*-cymene, 7 % γ -terpinene, and 4 % linalool and exhibited the strongest antimicrobial effect. Although none of the tested oils surpassed *T. vulgaris*, several showed notable antimicrobial activity, including *Aniba rosaeodora* (86 % linalool, 4 % eucalyptol), *Pimenta officinalis* (72 % eugenol, 13 % methyl eugenol, 8 % caryophyllene (E)), *Eugenia caryophyllata* (85 % eugenol, 7 % eugenyl acetate, 6 % caryophyllene (E)), and *Evernia prunastri* (100 % methyl atrarate).

Our results suggest that essential oils hold unexplored potential as antimicrobial agents. Future research should explore the effects of individual essential oil components, synergistic interactions, and their potential use in combination with antibiotics.

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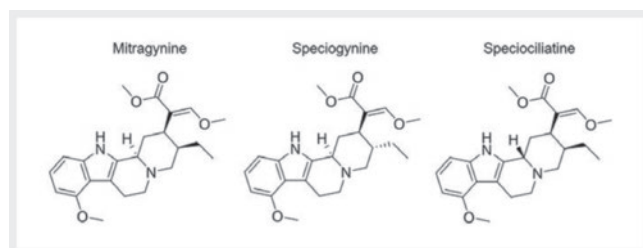
P268 Alkaloid extraction optimization from *Mitragyna speciosa* (kratom): A methodological evaluation

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Mitragyna speciosa, commonly known as kratom, is a psychoactive plant in the Rubiaceae family. Traditionally, the fresh leaves of *Mitragyna speciosa* have been consumed in Southeast Asia for their therapeutic properties for centuries. Kratom has been used to relieve pain, reduce opioid use and relieve withdrawal, and increase sexual desire. Phytochemical and pharmacological studies have been prompted by its unique pharmacological properties due to its rich alkaloid content. In continuation of our previous investigation on alkaloids [1, 2], the present study was focused on the evaluation of the alkaloid yield and composition in kratom extracts. Dried *M. speciosa* leaves were treated using various extraction techniques including maceration, ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE) and supercritical carbon dioxide extraction SFE-CO₂, using variety of solvents mostly methanol, propan-2-ol, water and binary mixtures. Quantitative analysis of the extracts was carried out using HPLC-MS-PDA, supported by calibration curves constructed for representative indole alkaloids specifically mitragynine, 7-hydroxy mitragynine, mitraphylline, speciogynine and speciociliatine (► Fig. 1). In addition, the present work includes a comparative investigation of recovery methods for alkaloids, employing solid-phase extraction, macroporous resin and acid base extraction. Our study demonstrates that extraction techniques significantly impact the yield and chemical profile of alkaloids obtained from plant material, directly influencing the potency and effectiveness of downstream products.



► Fig. 1 Structure of mitragynine, speciogynine and speciociliatine

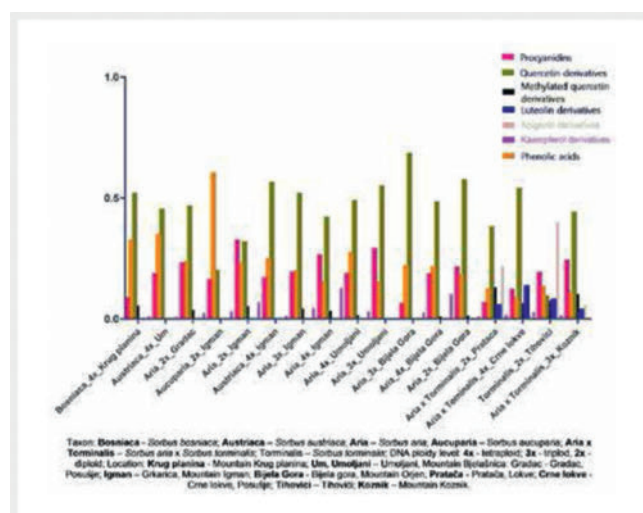
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P269 Polyphenolic diversity in *Sorbus*: chemotaxonomic approach to hybridization and secondary metabolite production

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The genus *Sorbus* exhibits considerable diversity due to processes such as hybridization, polyploidy, autopolyploidy and apomixis, which also influence the production of polyphenolic compounds [1, 2]. Our previous study showed the chemotaxonomically significant connection between flavonoids and hybridization [3]. To achieve a comprehensive chemotaxonomy of *Sorbus*, a detailed phytochemical analysis of procyanidins and phenolic acids was performed. Leaf material was collected from *Sorbus* accessions at ten locations in Bosnia and Herzegovina, representing both the parent species (*S. aria*, *S. torminalis*, *S. aucuparia*) and their hybrids (*S. aria* × *S. torminalis*, *S. aria* × *S. aucuparia*). Besides 23 flavonoids [3], a total of 7 phenolic acids and 5 procyanidins were identified using the LC-MS method based on UV spectral data, MS fragmentation patterns and commercially available standards. Chlorogenic acid (0.455–5.204 g/100 g dry extract) and 1,5-*O*-dicafeoylquinic acid (0.112–1.998 g/100 g dry extract) were the most abundant. The most abundant procyanidins were type B trimers (0.464–6.334 g/100 g dry extract) and dimers (0.224–2.509 g/100 g dry extract), with a type B procyanidin trimer found specifically in the endemic *S. bosniaca* and its parent species *S. aria*. Of note, the B-type procyanidin dimer was not present in hybrids of *S. aria* × *S. torminalis*. Chlorogenic acid and caffeoylquinic acid were present in all samples, with neochlorogenic acid being specific only for the parent species of the *Tormaria* group, *S. torminalis*. The results suggest that each polyphenol group undoubtedly has an impact on chemotaxonomy and the relationships between hybridization, polyploidy and secondary metabolites production (► Fig. 1).



► Fig. 1 The relative content of phenolic acids, procyanidins, and flavonoids (classified according to their structure) in the ethyl acetate fractions of the studied *Sorbus* accessions.

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P270 Ellagic tannins and flavonoids in the leaves of two varieties of garden strawberry, *Fragaria x ananassa*

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The genus *Fragaria* L., according to the current taxonomy, includes at least 23 species of strawberry, which are distributed on all continents except Australia. Traditionally, *Fragariae folium* (*Fragaria vesca*, *F. moschata*, *F. viridis* Weston, *Fragaria x ananassa*, Rosaceae) is used as a herbal tea for the treatment of mild diarrhoea and as diuretic [1].

The aim of this study was to analyse the chemical composition of two varieties of the garden strawberry *F. ananassa* (octaploid), an Italian variety *Irma* and a Californian variety *Monterey*. The ripening period of both varieties starts in mid-May and lasts until October, with several harvest peaks [2]. Previous studies of the garden strawberry, *Fragaria x ananassa* (Duchesne ex Weston) Duchesne ex Rozier, confirmed the presence of anthocyanins and fisetin in the fruits [3, 4] and minerals in the leaves [5]. The chemical analysis of the methanol extracts was performed using LC-MS analysis and spectrophotometry. The galloylquinic acid, gallic/ellagic tannins and quercetin/kaempferol glycosides were detected in both varieties: caffeoyl-*O*-hexose, two HHDP(hexahydroxydiphenic acid)-hexosides, pedunculagin I isomers, galloyl-HHDP-DHHDP-hexose, galloyl-HHDP-hexoside, taxifolin-*O*-hexoside, isoquercetin, rutin, ellagic acid pentose, agrimoniin, kaempferol glucuronide and tilliroside. Ellagic acid *O*-deoxyhexoside was only detected only in the Monterey variety. The spectrophotometric analysis showed the following contents of: total polyphenols (*Irma*:

2.44 %; Monterey: 2.11 %); tannins (*Irma*: 1.68 %; Monterey: 1.17 %), flavonoids (*Irma*: 0.041 %; Monterey: 0.071 %); anthocyanins (*Irma*: 0.009 %; Monterey: 0.007 %) and procyanidins (*Irma*: 0.51 %; Monterey: 0.22 %).

Our results are only partly in accordance with the composition of *F. vesca* leaves which are rich in both ellagic and condensed tannins.

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P271 Chemical analysis, cytotoxicity and enzyme inhibitory activities of three *Alchemilla* species

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The genus *Alchemilla* L. (Rosaceae) is a rich source of flavonoids, tannins, and phenolic acids. Ethnobotanical studies on *A. vulgaris* have shown different therapeutic properties, from female problems, such as dysmenorrhea, menorrhagia, pruritus vulvae and menopausal complaints, to the treatment of acute diarrhea, wounds, hypertension and diabetes. Researches showed anti-inflammatory, antimicrobial, anticancer, antioxidant and other activities of different *Alchemilla* species [1] and, for example, ACE inhibitory activity and inhibition of SARS-CoV-2 internalization *in vitro* and *in silico* for *A. viridiflora* [2, 3].

The aim of our research was the chemical analysis of methanol extracts of herbs of *A. monticola*, *A. glabra* and *A. venosula* using LC-MS, their cytotoxicity against HeLa (human epithelial cervix adenocarcinoma), MRC5 (human normal lung fibroblasts) and NCI-N87 (human gastric carcinoma) cell lines cell lines, as well as the activities against α -amylase and α -glucosidase.

LC-MS analysis leads to the identification of 26 compounds in the methanol extracts, mainly ellagitannins and related compounds (pedunculagin isomers, sanguin H-10 isomers, tellimagrandin 1 and 2, galloyl-HHDP hexose etc.) and glycosides of quercetin and kaempferol. All methanol extracts have shown activity against the HeLa cell line (IC_{50} = 99–147 μ g/mL), without cytotoxicity against MRC5 and N87 cells. The activities against enzymes were: α -amylase, IC_{50} = 3,1–3,9 mg/mL, and α -glucosidase, IC_{50} = 2,5–3,6 mg/mL.

Given that most of the research is related to *A. vulgaris*, which represents the aggregate, our results represent a significant contribution to the study of the chemical composition and activity of other *Alchemilla* species, including *A. monticola*, which belongs to this aggregate.

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P272 Comparing computed and experimental NMR data for elucidating the structural and stereochemical features of organic compounds

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The comparison of experimental and computed NMR parameters (e.g., chemical shifts, *J* coupling constants) successfully aided the elucidation of the structural and stereochemical features of natural and synthetic compounds in a rapid and robust way.

This approach [1, 2] is based on a general workflow involving different steps: 1) building of the 3D input structure(s), e.g., all the possible isomers of the molecule under investigation; 2) conformational search rounds; 3) geometry optimization of the sampled conformers; 4) computation of NMR data, considering the influence of each conformer on the total Boltzmann distribution considering the relative energies computed at the density functional theory (DFT) level; 5) comparison of experimental and predicted data. [1, 2] Among them, step 4) usually features a high source of potential drawbacks dramatically affecting the final results. Indeed, DFT often shows limitations in correctly reproducing the conformational dynamics of organic compounds by overestimating improper conformations, e.g., those showing intramolecular H-bonding. Different strategies have been proposed to solve this problem, e.g., combining several computed/experimental NMR parameters and redefining the Boltzmann distribution by stochastic approaches.[3]

To deeply investigate this aspect, we developed a computational tool based on systematically modulating the relative weight of the conformers from the original Boltzmann distribution, building a number of “artificial” ensembles each accounted for the comparison of experimental/predicted data. This tool highlighted promising results for several challenging organic compounds, focusing on cases for which the structural elucidation was difficult or misleading, e.g., highly flexible and/or polyhydroxylated molecules.

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P273 Discovery of new secoiridoids in *Ligustrum japonicum* By-products via GNPS-molecular networking

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Ligustrum japonicum Thunb. (Oleaceae) has traditionally been valued for the medicinal properties of its fruits, whereas its leaves and branches are generally discarded as post-harvest by-products, with limited phytochemical investigation [1]. To assess the potential of these underutilized parts, we conducted a comparative metabolomic analysis of the fruits, leaves, and branches using HPLC-MS/MS coupled with molecular networking on the GNPS platform. The analysis revealed both shared and distinct metabolite profiles among the three plant parts. Chromatographic separation yielded 14 secoiridoid derivatives common to all parts. Furthermore, four previously unreported secoiridoids were isolated and structurally elucidated via spectroscopic techniques: secoligunosides A (1) and B (2) from the leaves, and secoligunosides C (3) and D (4) from the branches. Among the major secoiridoids identified, oleuropein (10), 8Z-nuezhenide (17), and GL-3 (18) induced weak proliferative effects on human dermal papilla cells. These findings suggest that the leaves and branches of *L. japonicum* not only retain bioactive compounds found in the fruits but also produce unique metabolites, underscoring their value as alternative sources of phytochemicals. This study highlights the potential of *L. japonicum* by-products as promising candidates for future therapeutic development.

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P274 To make tea or not to make tea-that is the question?

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In South Africa, the pepper bark tree or *Warburgia salutaris* () is used as an important part of traditional medicine. Since *W. salutaris* is endangered, substituting leaves with bark has been proposed as a more sustainable way of using the plant parts. This study aimed to make teas with various ratios of bark to leaves to see if this was a feasible way of reducing bark usage (combinations use less bark) and if the antimicrobial activity (minimum inhibitory concentrations and antibiofilm activity) was comparable. The results demonstrated that the best activity (MIC 0.31–0.62 mg/mL) was seen for the bark to leaves combination (75:25) for both reference and clinical *P. aeruginosa* strains, the *L. monocytogenes* reference strain and *C. albicans* reference strains. Strong antibiofilm activity of the teas of *W. salutaris* was observed against 24 hr preformed *C. tropicalis* biofilm (81.5–97.4%) for the various ratios of bark to leaves. These findings demonstrate that teas using only leaves or leaves in combination with bark material should be further explored. Teas offer an alternative use of plant parts and could contribute to more sustainable use of the plant parts thus promoting conservation of this tree without compromising on the reported antimicrobial activity [1].

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P275 Establishment and antifungal potential of *in vitro* biomass of *Salvia somalensis*

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Carnosic acid, a well-known abietane diterpenoid produced by several *Salvia* spp., including *S. somalensis*, has shown potential as an agrochemical for the treatment of several phytopathogenic fungi [1]. Aim of this project was to establish micropropagated plants from *S. somalensis*, to assess their production of carnosic acid and to investigate the antifungal activity against common plant pathogens. Explants placed in medium containing the growth regulators, 0.5 mg/L BA plus 0.1 mg/L NAA, produced an average of 7.43 shoots per explant. The methanolic extracts of the aerial parts and micropropagated plants (MP) of *S. somalensis* were characterized by LC-MS analysis. Both produced carnosic acid, as well as carnosol, 12-methylcarnosic acid, 20-deoxocarnosol, and other terpenoids. The antifungal activity of the two extracts was assessed against *Rhizoctonia solani*, *Fusarium solani*, *Colletotrichum coccodes*, *Sclerotinia sclerotiorum*, and *Botrytis cinerea*. The extract of the *in vivo* biomass was active against all the fungi tested. The activity at 1000 µg/mL against *F. solani*, *B. cinerea*, and *C. coccodes* (72%, 84%, and 86% inhibition of mycelial growth, respectively) was statistically comparable to the activity of the reference commercial fungicides Ortiva® and Switch® (Syngenta), applied at 1000 µg/mL. The extract of the MP at 1000 µg/mL was active against *B. cinerea*, *R. solani*, *C. coccodes*, and *S. sclerotiorum* (63%, 69%, 63% and 62% inhibition of mycelial growth, respectively).

Although the antifungal activity of the MP was statistically lower than the *in vivo* biomass, the results confirmed the significance of MP as a source of bioactive compounds of agricultural interest.

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P276 Bioactive compounds in the roots and fruits of *Withania somnifera* L.-Chemical and biological analysis of plant from polish cultivations

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Withania somnifera L. (Ashwagandha) is a medicinal plant known for its adaptogenic, anti-inflammatory, and neuroprotective properties, traditionally used to enhance stress resilience, support cognitive function, and improve overall vitality. The roots and fruits of *W. somnifera* contain numerous bioactive compounds, including withanolides, alkaloids, flavonoids, and phytosterols. Withanolides, found mainly in the roots, exhibit strong adaptogenic, anti-inflammatory, and immunomodulatory effects. The fruits also contain antioxidants that help protect cells against oxidative stress. The main objective of the project was to analyze the phytochemical composition and evaluate the biological properties of the fruits and roots of *Withania somnifera* cultivated under Polish climatic conditions. Extracts from *W. somnifera* fruits obtained through different extraction methods show significant differences in biological activity and

chemical composition. Maceration provides the highest concentration of active compounds and the greatest effectiveness in inhibiting acetylcholinesterase and antioxidant activity. Boiling demonstrates moderate efficiency, while ultrasound-assisted extraction (UAE) has the lowest bioactivity despite achieving the highest extraction yield. The results obtained for *W. somnifera* root extracts were stronger than those for the fruits, both in terms of acetylcholinesterase inhibition and antioxidant activity. The higher biological activity of the roots indicates a greater concentration of bioactive compounds [1, 2].

The extraction of *W. somnifera* shows significant differences depending on the method and raw material used, with the roots exhibiting stronger biological activity and a higher concentration of bioactive compounds than the fruits, which may influence their potential therapeutic applications.

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P277 Solvent-free synthesis of chalcones for enzymatic biotransformation reactions

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The use of biotransformation through enzymatic fungal secretomes has proved to be an effective strategy for generating new molecules from readily available natural products [1–4]. After applying this methodology to stilbenes [1–4], we were able to generate a series of compounds with particularly interesting structures from chalcones. Based on the reaction mechanisms observed during the synthesis of these compounds, additional chalcones were synthesized with phenol substitutions to enhance reaction selectivity. A green chemistry approach, a solvent-free Claisen-Schmidt condensation reaction [5] was used to achieve this. It is cost-effective, energy-efficient, and often results in higher yields, improved selectivity, and shorter reaction times. The reactions allow real-time monitoring using nuclear magnetic resonance (NMR), which facilitated precise optimization of the conditions. The newly synthesized chalcones were purified by flash or semipreparative chromatography and subsequently biotransformed using laccases from *Trametes versicolor*. The biotransformation mixtures were purified by high-resolution preparative chromatography in combination with a dry-load technique. Full de novo structure elucidation was achieved for all biotransformed compounds. The resulting structures confirmed the hypothesized reaction pathways, which were based on phenoxy radical coupling mechanisms. The antimicrobial activity of the newly generated compounds was then evaluated against Gram-positive and Gram-negative bacteria. This approach exemplifies the integration of green synthetic methods and enzymatic biotransformation to expand chemical diversity and explore bioactive properties in drug discovery pipelines.

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P278 Quantification of natural compounds in medicinal plant extracts: a case study on the leaf extract of *Punica granatum* L.

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The identification and quantification of natural products is laborious, due to the complexity of plant extracts and the limited availability of pure reference standards [1]. This study aims to evaluate the precision of plant metabolite quantification in high performance liquid chromatography (HPLC) coupled with different detectors (PDA and MS), focusing on semi-quantification which occurs when the reference standard is not available. As a case study, an extract from *Punica granatum* L. leaves was selected. Although these non-edible plant parts are usually discarded, they have been proven to be a valuable source of bioactive metabolites. Their traditional medicinal use is documented, and *in vitro* studies have demonstrated beneficial effects, including inhibition of Zika virus infection and HIV-1 reverse transcriptase and integrase [2–4]. In this work the performance of PDA and MS in different acquisition modes (SIM and SRM) is compared for the quantification of luteolin 4'-O-glucoside, a major compound of the extract [1, 2]. Quantification precision was calculated as percentage relative error, comparing the results with the calibration curve generated with either the reference standard or a similar compound, i.e. kaempferol 3-O-glucoside. As expected, quantification with the reference standard is more accurate, while semi-quantification gives higher percentage relative errors, especially with MS, due to differences in compound responses. To address this issue, a correction method using a correction factor is proposed to compensate for the over- and underestimation in semi-quantification. This approach led to promising results.

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P279 Light-modulated wound healing activity of four traditional Chinese medicinal plants: A comparative study

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Wound healing has gained growing attention due to the increasing burden of chronic and infectious skin injuries [1]. Plant-based medicines have long been used for their multi-target regulatory effects, low side effects, and accessibility. Photodynamic therapy (PDT), which employs photosensitizers to transfer light energy to cellular oxygen to produce reactive oxygen species (ROS), has shown promise in dermatology. However, studies combining medicinal plant extracts with PDT for wound treatment remain limited [2, 3].

The wound healing activities of four methanol extracts from plants documented in the Chinese Pharmacopoeia—*Lobelia chinensis* Lour, *Cnidium monnieri* (L.) Cusson, *Bistorta officinalis* Delarbre, and *Rubia cordifolia* L.—were assessed, and the potential of visible light (400–700 nm) exposure to enhance their effects was investigated. Extracts were tested on HaCaT cells for cytotoxicity (SRB), migration (scratch assay), and antioxidant activity (DCFH-DA) under light and dark conditions.

Light exposure significantly increased the cytotoxicity of *L. chinensis* and *C. monnieri*. *L. chinensis* inhibited cell migration in darkness but promoted it under light at 10 µg/mL. *C. monnieri* and *B. officinalis* enhanced migration under both conditions, with optimal effects at 50 µg/mL. *R. cordifolia* consistently inhibited migration, particularly with light. Light generally increased intracellular ROS, and except *L. chinensis*, rest extracts reduced H₂O₂-induced ROS to varying degrees.

In conclusion, these plant extracts have shown the potential for wound healing through different mechanisms. In some cases, visible light enhanced their activity, suggesting that combining plant extracts with PDT could offer a promising therapeutic approach. Further mechanistic and chemical studies are warranted.

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P280 Unlocking Nature's Pharmacy: Phytochemical investigation of *Succisa pratensis*

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Succisa pratensis, or devil's-bit scabious, is a member of the Caprifoliaceae family and found across Europe, western Siberia, and North Africa. Traditionally, it has been used in the British Isles to treat rheumatism, bruises, toothache, throat sores, dog bites, and scurvy, and in Ireland, it is particularly employed for treating skin problems like boils [1]. Previous studies have shown the plant's diverse pharmacological potential, including antibacterial, antifungal, antioxidant, anti-inflammatory, and antiproliferative activities, especially in the methanol-

ic extract of its leaves. Additionally, *S. pratensis* extract have been reported to reduce NF-κB activation and lower NF-κB and COX-2 gene expression in HepG2 cells [2–4]. Although some phytochemical analyses using LCMS and GCMS have been conducted, limited work has been done to isolate and characterise its active constituents. In this study, the methanolic extract of *S. pratensis* leaves was fractionated using chromatographic techniques, resulting in the isolation of 10 compounds, including scopoletin, scopolin, chlorogenic acid, and various dicaffeoyl quinic acid derivatives – 3,5-dicaffeoyl quinic acid, 3,4-dicaffeoyl quinic acid, 3,5-dicaffeoyl quinic acid methyl ester, 3,4-dicaffeoyl quinic acid methyl ester, along with secologanin and gentiopicoside. Structural confirmation was achieved through mass spectrometry and NMR spectroscopy. Notably, scopolin, secologanin derivatives, and dicaffeoyl quinic acid derivatives were isolated and characterised for the first time in *S. pratensis*. These findings contribute to the exploration of native Irish medicinal plants for bioactive compounds and enhance the understanding of *S. pratensis*'s chemical constituents, highlighting its potential for future pharmacological applications.

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P281 Phytochemical investigation and anti-inflammatory potential of *Erica cinerea*

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Erica cinerea (bell heather), a member of the Ericaceae family, is native to central and western Europe and produces secondary metabolites such as anthocyanins, coumarins, flavonoids, and diarylnonanooids [1, 2]. While some studies highlight its potential as a urinary antiseptic and diuretic, its traditional use for inflammatory conditions remains underexplored [3]. As part of the 'Unlocking Nature's Pharmacy from Bogland Species' (UNPBS) project, we have investigated the anti-inflammatory potential of *E. cinerea* using a bioactivity-guided approach. Initially, a crude methanolic extract of *E. cinerea* was prepared and tested for cell viability and its effects on IL-6, Rantes, TNF-α, and IL-1β using the THP-1 cell line stimulated with LPS, which yielded promising results. The extract was then fractionated using multiple chromatographic techniques, and the resulting fractions were evaluated for cell viability and anti-inflammatory activity. The most active fraction (EA 2.2) was subjected to LCMS, GCMS and HPTLC based profiling and finally four major compounds were isolated utilising column chromatography. The compounds were structurally characterised by NMR and mass spectrometry and identified as scopoletin, protocatechuic acid, ursolic acid and 3''-hydroxy anhydroericanone. Of these, the compound 3''-hydroxy anhydroericanone significantly suppressed LPS, PAM3CSK4, and IL-1β induced cytokine release, while the remaining three compounds showed minimal activity. Mechanistic studies indicated that 3''-hydroxy anhydroericanone inhibited LPS induced NF-κB phosphorylation, suppressed NF-κB reporter activity, and reduced JNK and p38-MAPKs phosphorylation. This research emphasises the potential of *E. cinerea* extract, particularly 3''-hydroxy anhydroer-

icanone, a diarylnonanoid as the potential therapeutic candidate for the treatment of inflammatory conditions.

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P282 Propolis as a natural therapeutic agent for atopic dermatitis via MKK4 pathway regulation

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Propolis, a natural resinous compound synthesized by bees from various botanical sources, is widely recognized for its diverse bioactive properties and potential as a functional food. Despite its known benefits, the therapeutic effects of propolis on atopic dermatitis (AD) have not been fully elucidated. This study aimed to evaluate the anti-AD efficacy of propolis, identify its active component, and uncover its molecular target. In TNF- α -stimulated HaCaT keratinocytes, propolis significantly suppressed the secretion of pro-inflammatory cytokines IL-6 and IL-8, as well as chemokines such as MCP-1 and MDC. It also restored the expression of skin barrier proteins, including filaggrin and involucrin. Similar modulatory effects were observed in an AD-like human skin model. In vivo, oral administration of propolis in DNCB-induced AD mice alleviated clinical symptoms, enhanced skin barrier function, reduced scratching behavior, and decreased transepidermal water loss (TEWL). Propolis also normalized the expression of AD-related genes in dorsal skin tissues. Through comparative analysis of its major constituents, caffeic acid phenethyl ester (CAPE) was identified as the principal bioactive compound. Further mechanistic studies demonstrated that both CAPE and propolis directly and selectively target MKK4. Collectively, these findings highlight the therapeutic potential of propolis as a functional food-based intervention for atopic dermatitis [1, 2].

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P283 Naviglio Extractor®: extracts from medicinal plants to produce supplements and beverages in short times (2-24 hours). Presentation of a new bitter: “Amaro delle Donne”

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The current industrial production of most extracts from medicinal plants is mainly based on traditional extraction techniques such as infusion, also improperly called “maceration”, squeezing and percolation; these methods, although economical and simple to apply, have numerous limitations. Among these, the best known is the long extraction time needed to obtain the final product that can cause the degradation of the matrices due to the long contact between the solvent and the solid [1]. The aim of this study is to summarize the last twenty-five years of development of an innovative solid-liquid extraction technique: the Rapid Solid-Liquid Dynamic Extraction (RSLDE), which uses the associated machinery, the Naviglio Extractor® (Atlas Filtri s.r.l., Padua, Italy) and to show its application in the production of extracts from different plant matrices in a shorter time than traditional methods, maintaining high quality standards and high yields in the beneficial compounds present in numerous medicinal plants [2, 3]. The most notable examples are the production of elixirs, which normally require up to 40 days of maceration, in just 24 hours of extraction [4] and the production of the traditional Neapolitan lemon liqueur called “Limoncello” in only 2 hours, as an alternative to the common 7-21 days of maceration required [5], showing the potential of this technique to revolutionize the extraction industry. Furthermore, the versatility of this technique allows its application to a multitude of plants listed in the International Pharmacopoeia, reducing the range of extraction times from 21 days to 2-24 hours (► Fig. 1).



► Fig. 1 Applications of Naviglio Extractor®

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P284 *Veronica agrestis* L.: free volatile compounds from wild and cultivated species

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The emission of free volatile compounds into the atmosphere is made up of anthropogenic and natural sources. The anthropogenic impact on the atmosphere is negative and warming has various effects on isoprene molecule and alteration of biosynthetic pathways [1]. The investigated species *Veronica agrestis* L. (Plantaginaceae) is an edible and medicinal plant used in ethnobotany [2, 3]. The compositions of the extracts, are compared between plants collected in their natural habitat (NH) and cultivated species (CS). The extracts were isolated by microwave-assisted extraction, and each extract obtained consists of a lipophilic (essential oil, EO) and an aqueous phase (hydrosol, HY). Composition of the isolates was determined by gas chromatography-mass spectrometry. The NH EO of *V. agrestis* consisted of 17.46% hexahydrofarnesyl acetone, and in CS EO the same compound was identified at 29.58%. Phytol was detected significantly more in extracts from the natural habitat (18.76%) than in those from cultivation (6.39%). The composition of CS HY is dominated by α -thujene, benzaldehydes and (*E*)- β -damascenones, each of which has a percentage share of more than 14%. These differences in the composition of extracts confirm the influence of environmental factors on metabolic pathways. Therefore, these extracts show different biological activities. Antioxidant activity was investigated with two methods, ORAC (oxygen radical absorbance capacity) and β -carotene bleaching test. Results showed that ORAC activity of NH EO was higher than from CS EO. For hydrosols, there was no significant differences between extract in ORAC method but higher activity was found in NH HY for β -carotene bleaching test.

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P285 Investigation of the phytochemical content and the antioxidant activity of the endemic plants of Cyprus *Alyssum troodi* and *Scutellaria cypria* subsp. *cypria*

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Alyssum troodi and *Scutellaria cypria* subsp. *cypria* are both endemic plants of Cyprus, located on Troodos mountains and belongs to Brassicaceae and Lamiales respectively, which are known for their nutraceutical and pharmaceutical properties [1]. In this study, aboveground parts of species were collected during their flowering stage from populations grown at 1790 m altitude. Flowers were separated from stem-leaves, cryopreserved, lyophilized, pulverized, and stored at -80 °C. The main aim was to investigate the antioxidant activity and the phytochemical content of plant extracts. Crude extracts derived using hydromethanolic solvents, at concentrations of 60%, 80%, and 100% methanol. Furthermore, the effect of homogenization on extraction efficiency was evaluated. Antioxidant activity was examined using *in vitro* assays (DPPH, FRAP). DPPH indicated that antioxidant activity increased with decreasing water content in the solvent with non-homogenized stem-leaves extracts exhibiting the highest activity. Conversely, FRAP assays indicated peak antioxidant activity in 60% methanolic extracts. Phytochemical analysis demonstrated that 60% methanolic extracts yielded the highest total phenolic content (TPC), while 80% methanolic extracts yielded the highest total flavonoid content (TFC).

Non-homogenized stem-leaf samples of both species exhibited elevated levels of these phytochemicals. Notably, *A. troodi* exhibited superior antioxidant activity and higher concentrations of TPC and TFC compared to *S. cypria* subsp. *cypria*. Overall, the stem-leaf tissues of both species represent promising sources of bioactive compounds. Solvent polarity significantly influenced extraction efficiency, whereas homogenization had a minimal effect on the overall yield. A strong positive correlation was observed between antioxidant activity and the levels of TPC and TFC.

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P286 β -caryophyllene mitigates oxidative stress, inflammation and fibrosis by activating cannabinoid-2 receptors in murine model of high-fat diet/streptozotocin-induced nonalcoholic fatty liver disease

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Non-alcoholic fatty liver disease (NAFLD) is a clinicopathological condition, frequently arising as a complication of type 2 diabetes mellitus (T2DM), and is characterized by hepatic steatosis. NAFLD has become a significant global public health concern. Its progression involves a complex interplay of lipid accumulation, oxidative stress, inflammation, and fibrosis. The condition is strongly linked to central obesity, dyslipidaemia, hypertension, hyperglycaemia, and altered liver function tests. Currently, the primary treatment strategy centers on weight reduction through lifestyle and dietary modifications. Growing evidence highlighting the role of dietary cannabinoids in liver diseases and diabetes that led to investigations into β -caryophyllene (BCP), a naturally occurring cannabinoid type-2 (CB2) receptor agonist in NAFLD. This study investigated the effects of BCP in a mouse model of NAFLD induced by a high-fat diet (HFD)/streptozotocin (STZ) administration. Following onset of diabetes, mice received a 12-week oral BCP (50 mg/kg). BCP reduced liver injury markers, mitigated hepatic oxidative stress by inhibiting the expression of NADPH oxidase 2 and NADPH oxidase 4 and activating Nrf2 signaling. BCP also inhibited inflammation and down-regulated inflammasome activation and suppressed hepatic fibrosis and endothelial-to-mesenchymal transition mediating inhibition of TGF- β /Smad signaling. To elucidate CB2R-dependent mechanism of BCP, T2DM mice were pretreated with AM630, a CB2R antagonist. Pharmacological challenge with AM630 abrogated the beneficial effects of BCP in T2DM mice. Taken together, BCP improved hepatic impairment efficiently in diabetic mice by suppressing oxidative stress, inflammation, and fibrosis mediating CB2R-dependent mechanisms. These results suggest that CB2R activation may represent a novel therapy for NAFLD with diabetes.

P288 Diosgenin suppresses angiogenesis via ROCK1 inhibition during anti-rheumatic therapies of *Dioscorea spongiosa*

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Rheumatoid arthritis (RA) is the most common autoimmune disease, affecting 0.5%–1% of the total population worldwide. It is characterized by persistent inflammation and progressive injuries in multiple joints, and will eventually result in disabilities without appropriate medical interventions [1, 2]. The rhizome of *Dioscorea spongiosa* is used as a Chinese herbal medicine Bixie (BX) to treat RA. This study investigated the mechanism behind the anti-angiogenesis effects of BX. Adjuvant-induced arthritis (AIA) rats were orally treated with BX for 28 days. The therapeutic efficacy of BX was validated by serological indicators tests, histological and immunohistochemical observation. Metabolic changes were analysed by kits-based metabolites quantification and UPLC-MS analyses. The components in BX, fractions and blood from BX-treated rats were identified by LC-MS. Protein targets were pulled down by biotin-tagged compounds. Their interaction was tested by simulation docking, immunoprecipitation, thermal shift assays and enzyme digestion. BX extract eased inflammatory cytokines increase, synovial invasion and metabolic alteration in AIA rats. Angiogenesis in both white adipose tissues and joints were attenuated as demonstrated by reduced vessels, pathological factors and CD31 expression. BX inhibited glycolysis in HUVEC cells and impaired their angiogenic ability. The expression of genes/proteins involved in VEGF and glycolytic pathways were down-regulated. Being an abundant compound in multiple bioactive fractions, diosgenin showed potentials in suppressing the activation of HUVEC cells. Biotin-conjugated diosgenin selectively bound to ROCK1, leading to its stabilization and phosphorylation inhibition. In conclusion, diosgenin inhibits ROCK1 and consequently suppresses angiogenesis dependent of glycolysis down-regulation.

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P289 Biomimetic synthesis of oligomeric stilbenoids as dental biomaterials

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Stilbenoids are a structurally complex class of phenolic oligomers with promise as dental biomaterials. While oxidative coupling of resveratrol has been explored using AgOAc, FeCl₃, K₃[Fe(CN)₆], and MnO₂, targeted and regioselective synthesis remains challenging. Systematic evaluation and optimization of reaction conditions, including solvent effects (MeOH, THF, Me₂CO), temperature, and reaction time, now enabled selective generation of dimeric to tetrameric stilbenoids. The products including their stereochemistry were characterized comprehensively by NMR and LC-ESI-HR-MS. Further modifications via regioselective hydrogenation using Pd/C yielded stable, saturated analogues of ϵ -/ δ -viniferin and vitisins A + B with >90% efficiency. Preliminary evaluations indicate that these resveratrol-derived stilbenoids enhance the mechanical properties of dentin in a structure-dependent manner, highlighting their potential for dental material applications. This study establishes a synthetic framework for accessing bioactive resveratrol oligomers with controlled structural diversity, thereby advancing their potential for material and biomedical applications [1, 2].

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P290 Optimizing phenolic content and antioxidant activity in cardoon extracts through biostimulant application

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Cynara cardunculus var. *altalis* DC., a Mediterranean plant of the Asteraceae family, is traditionally consumed as a vegetable and valued for its fiber content. Beyond its agronomic role, cardoon is rich in bioactive compounds, particularly phenolics, which contribute to the hepatoprotective, antidiabetic, antioxidant, anti-inflammatory, antimicrobial, and antifungal properties [1, 2]. This study investigates the effect of two biostimulant formulations, protein hydrolysate (PH) and algae-based solution (SW), on phenolic content and antioxidant activity of cultivated cardoon extracts. The genotype cv "Bianco Avorio" (La Semio Sementi, Sarno, Italy) was selected for its high productivity and adaptability to floating systems [3], a technique that eliminates issues related to soil presence and allows for more precise control of nutrients. Plants were cultivated in floating raft culture in the greenhouses of "Nello Lupori" Experimental Farm, Tuscia University. Physiological parameters such as chlorophyll, anthocyanins, and flavonols were monitored throughout the cultivation period and five sampling events conducted. Extracts were obtained via maceration and by microwave-assisted extraction with an ethanol/water solution. Total phenolic content (TPC) was determined using the Folin-Ciocalteu method, while antioxidant activity was evaluated via the DPPH assay. Experimental results showed that PH-treated plants showed an increase in TPC compared to both SW-treated plants and untreated. Similarly, DPPH assay confirmed higher antioxidant activity in PH-treated extracts. These findings suggest that the application of the biostimulant PH can permit the production of extracts with distinctive features compared to others, an added value that can enhance their potential use in medicine and nutraceutical sector, but also for agricultural application.

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P291 Isolation of new sulfur-containing constituents from *Salvadora persica* leaves

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The facultative halophyte *Salvadora persica* L. (Salvadoraceae) is extensively used in traditional medicine of its African, Asian and Middle Eastern countries of origin, with applications related to oral health being most prominent for all plant parts. Flavonoids, phenylpropanoids, and volatile oil have been identified as the main classes of constituents occurring in *S. persica* leaves, while the main sulfur-containing compounds described so far were the glucosinolate glucotropaeolin and its myrosinase-reaction product benzylisothiocyanate [1]. In a recently performed metabolite profiling study, we were able to annotate a wide range of sulfur-containing constituents in *S. persica* leaf extracts [2]. The aim

of this study was to isolate and elucidate the structure of major yet unknown sulfur-containing constituents of *S. persica* leaves.

A methanolic *S. persica* leaf extract was subjected to liquid-liquid extraction followed by fractionation of the n-butanol subextract by vacuum liquid chromatography on silica gel and RP-18 material, anion exchange chromatography, preparative HPLC and preparative TLC.

Overall we were able to isolate and structurally elucidate five new megastigmane glycosylsulfates, among them 3'-O-sulforhododendroside and 9-epibulmenol C-9-(3'-O-sulfo)glucoside, together with 7,8-dihydro- β -ionone-3-(3'-O-sulfo)glucoside known from our previous study [2]. Furthermore, three new aromatic compounds were isolated, namely, 1-O-benzyl-3-O-sulfolucose, an isomer of the already known compound salvadoside [3], and 3'- and 4'-sulfo-prunasin, two isomeric sulfated cyanogenic glycosides.

Our study allows to add new, hitherto overlooked compound classes to the chemistry of *S. persica*. The pharmacological relevance of the newly identified compounds, in particular with regards to oral health-related effects, needs to be investigated in future studies.

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P292 Probing the immunomodulatory activity of *Sphagnum* spp. from Irish boglands

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Sphagnum species are mosses of the bryophyte taxonomic division, widely distributed in the Northern Hemisphere in wetland areas where accumulation of *Sphagnum* material forms peat bog habitats. In this study, we probed the *in vitro* immunomodulatory activities of methanolic extracts of *Sphagnum* spp. collected from raised bogland in county Offaly in the midlands of Ireland. Extracts of eight species were prepared by Soxhlet extraction and screened for immunomodulatory effects on lipopolysaccharide (LPS)-induced cytokine release in phorbol 12-myristate 13-acetate differentiated THP-1 cells. All *Sphagnum* spp. extracts tested significantly reduced the release of IL-6 and RANTES on stimulation, with complete attenuation of IL-6 by *S. capillifolium* and *S. papillosum* at 100 μ g/mL. These species extracts also demonstrated the most significant attenuation of RANTES observed. There was a trend to reduction of IL-1 β release by all treatments, *S. capillifolium* and *S. papillosum* again showing the most significant effects. In ELISA of TNF, pro-inflammatory effects were observed, with some treatments inducing significant TNF release in comparison to DMSO vehicle treated cells in the absence of LPS but TNF release induced by LPS was significantly reduced by the *S. capillifolium* and *S. papillosum* treatments. LPS triggers an immune response predominantly by binding Toll-like receptor 4 (TLR4) activating intracellular signalling cascades. Western blot analysis showed that *S. capillifolium* and *S. papillosum* treatments suppressed LPS-induced phosphorylation of both NF- κ B and JNK but not p38. The immunomodulatory effects of these extracts warrant further investigation and delineation of the contributing fractions, constituent or combination of constituents is underway.

P293 *Chamaemelum nobile* L.: Chemistry and evaluation of tyrosinase and acetylcholinesterase inhibitory activity

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The aging of the world population has led to an increase in the incidence of neurodegenerative diseases, which represents a still open challenge in the field of scientific research. In this regard, plants have become an important source of bioactive principles able to act on multiple targets [1]. *Chamaemelum nobile* L. is a perennial herb of the Asteraceae family, known as Roman chamomile, less studied in scientific literature than the more common *Matricaria chamomilla*, although it is also widely used in Traditional Medicines due to its antioxidant, mild sedative and antispasmodic properties [2]. In the present work, with the aim of evaluating the potential CNS activity of *C. nobile* L., different green extracts of *C. nobile* were tested for the antioxidant activity, and tyrosinase and acetylcholinesterase (AChE) inhibitory activity. Moreover, 15 compounds (coumarins, glucoside derivatives, flavonoids and sesquiterpenes lactones) were isolated from *C. nobile* and tested for tyrosinase and AChE inhibitory activity. The results showed that the highest activities were obtained for the 60% and 50% EtOH:H₂O extracts. Tested compounds presented interesting tyrosinase inhibitory activity (IC₅₀ values: 32.09–412.02 μ M) and, among the sesquiterpene derivatives, 11,13-dihydro-8-tigloylhydroxyisobornil, here reported for the first time, exhibited the highest activity. A kinetic study on this compound indicated an uncompetitive inhibition. All compounds showed AChE inhibition in a range of IC₅₀ values of 224.78–387.99 μ M.

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P294 *Lavandula dentata* L.: chemical composition and biological activity

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Lavandula dentata L. (Lamiaceae), also known as French lavender, is an ornamental, aromatic and medicinal plant. This species is used in traditional medicine as an antispasmodic, antidiarrhoeal, antilithiatric, antihypertensive and antirheumatic [1]. Recent findings have revealed the cytotoxic activity of *L. dentata* extract [2]. The aim of this project was to investigate the chemical composition of *L. dentata* leaf surface extract (LVD) and the bioactivity of the extract, fractions and isolates. In detail, LVD was obtained by rapid immersion of *L. dentata* leaves in dichloromethane and then separated by BIOTAGE flash chromatography to obtain simplified fractions. Phytochemical analysis of the LVD fractions led to the isolation and structural elucidation, by NMR and HRES-IMS data, of an undescribed lupane, a phytolic aldehyde, together with ten known triterpenes. Considering the chemical composition of LVD and the reported data on the antitumour potential of triterpenes [3], the cytotoxic activity of the extract was preliminarily tested using the crystal violet assay on HCT116 colon cancer cells. The results showed that the extract exhibited strong cytotoxic activity (~80% viability inhibition) at a concentration of 55.5 μ g/mL. Subsequently, seven LVD fractions were tested in HCT116 cells and non-tumour HaCaT cells. The viability of HCT116 cells was inhibited by all LVD fractions (0.1–55.5 μ g/mL) in a concentration-dependent manner. HaCaT cells treated

with LVD fractions were also sensitive, except for the LVD fraction, which is rich in lupane triterpenes and was not cytotoxic. Experiments are currently underway to identify the molecules responsible for the antitumour activity.

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P295 *Ptaeroxylon obliquum* acetone leaf extracts and obliquumol as potential apoptosis mediators in HepG2 and HeLa cells by caspase-3 and TNF- α

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In our previous investigation, obliquumol and *P. obliquum* acetone extracts had selective cytotoxicity against hepatocarcinoma (HepG2) and human cervical (HeLa) cancer cell lines. The aim of the study was to investigate the effects of the acetone extracts and obliquumol isolated from *P. obliquum* leaves from trees growing in different areas in Gauteng Province, South Africa on pro-apoptotic cytokines (caspase-3 and TNF- α) and cellular morphological alterations. The effects of the samples on levels of caspase-3 and TNF- α were determined and quantified using ELISA kits. Light microscopy was used to determine the morphological changes in HepG2 and HeLa cell morphology. *P. obliquum* acetone extracts and obliquumol increased caspase-3 production by 2% at 100 μ g/mL on HepG2 cells. Obliquumol increased the production of caspase-3 by 8% at 100 μ g/mL on HeLa cells. The acetone leaf extract (Hatfield) increased TNF- α production by ~10% at 100 μ g/mL in HepG2 cells which was higher than the positive control doxorubicin. Acetone leaf extracts (Roodepoort) increased TNF- α production by 11.5% at the lowest concentration tested (50 μ g/mL) on HeLa cells which was better than the positive control. A concentration-dependent effect was observed on the morphology of both cell lines tested in the study. Higher cell death and morphological alterations were observed on HepG2 cells than on HeLa cells. The *P. obliquum* acetone extract and isolated compounds may therefore contribute to the induction of pro-apoptotic cytokines (caspase-3 and TNF- α).

P296 Chemical profiling of *Rhododendron tomentosum* essential oils and phenolics during key growth stages

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Rhododendron tomentosum is an evergreen shrub which belongs to the family of Ericaceae. This plant is known to have a rich phytochemical composition and

accumulates essential oils, the composition of which varies according to scientific research [1]. The aim of this study was to evaluate the phytochemical composition of *R. tomentosum* during mass flowering and fruit ripening periods. The phenolic profile of *R. tomentosum* ethanol extracts was determined and included seven identified compounds: hyperoside, avicularin, quercetin, neochlorogenic acid, chlorogenic acid, catechin, and procyanidin A2. The quantities of the identified compounds varied between the samples collected during mass flowering and those collected during fruit ripening. Catechin (915.99 μ g/g) and procyanidin A2 (1216.28 μ g/g) were the dominant compounds in samples taken during the fruit ripening period. The other 5 compounds – hyperoside (863.21 μ g/g), avicularin (1050.39 μ g/g), quercetin (77.89 μ g/g), neochlorogenic (1613.06 μ g/g) and chlorogenic acids (1792.03 μ g/g) – were found in higher amounts in samples taken during mass flowering. Analysis of the essential oils revealed that *R. tomentosum* samples collected during mass flowering contained 54 identified compounds, while samples collected during the fruit ripening stage contained 56. At different phenological stages of the plant, the predominant compounds found were the same as α -terpinyl acetate, isoscaridol and p-cymene, but their quantitative values differed, constituting respectively 26.95%, 23.43% and 14.29% during mass flowering and 28.95%, 25.89% and 10.74% of the identified components during fruit ripening. The results revealed that the phytochemical composition of *R. tomentosum* varies with the phenological period of the plant.

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P298 Phytoconstituents, antioxidant capacity and antifungal activity of different species of the genus *Heliotropium* present in Chile

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Plants of the genus *Heliotropium* are widespread throughout the world and some of its species are part of traditional medicine for their antibacterial, antifungal and antitumor properties, attributable to the biochemical compounds that comprise them [1]. In Chile, it is possible to find different species of the genus *Heliotropium* in the north and center of the country, some of them have reported high concentrations of flavonoids in their resins [2]. These molecules can have different functions in plants, such as climatic, UV and antimicrobial protection [3]. Therefore, the resin of these plants becomes a topic of interest to solve problems such as phytopathogenic pests, antimicrobial resistance and diseases such as cancer. The aim of this research is to study the biochemical composition and biological activity of the resinous exudates of *Heliotropium sinuatum*, *Heliotropium glutinosum* and *Heliotropium stenophyllum*, performing tests to determine phytoconstituents, antioxidant capacity and antifungal activity. From the results, the resin of *H. glutinosum* obtained an IC₅₀ of 0.26 mg/mL in DPPH tests and the phenol content was 52.52 GAE mg/L (sample concentration of 0.5 mg/mL). The flavonoid content of *H. sinuatum* was 35.72 QE mg/L (sample concentration of 0.5 mg/mL). Antifungal activity was evaluated on *Monilinia fructicola*, where *H. stenophyllum* obtained effective concentration (EC₅₀) of 72.33 $\pm$ 0.61 μ g/mL. In conclusion, *H. sinuatum* and *H. glutinosum* indicate high antioxidant capacity that could be explained by their levels of phytoconstituents, in addition, the resinous exudate of *H. stenophyllum* shows potential antifungal activity, which could establish new therapeutic alternatives.

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P299 Immunomodulatory efficacy of an hydroethanolic extract from *Calendula officinalis* L. herba in vitro

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Calendula officinalis L. is traditionally indicated for the treatment of minor inflammations of the skin and as an aid in healing of minor wounds [1] that are physiologically accompanied by local inflammation, including activation of the NF-κB pathway and release of pro-inflammatory mediators such as leukotrienes and prostaglandins. Here we aimed to evaluate the anti-inflammatory efficacy of an hydroethanolic extract from *Calendula officinalis* L. herba.

Dry extracts from *Calendula officinalis* L. were prepared using liquid extracts from fresh aerial parts of the flowering Calendula plant (mother tincture *Calendula officinalis* 2a Ø (GHP)). NF-κB activation was analysed in a reporter assay with the human Jurkat cell line. Analysis of inhibition of the proinflammatory and pain-related enzymes 5-LOX and COX-2 as part of the arachidonic acid pathway was performed using recombinant human enzymes. Modulation of lipid mediator release was analysed in human M2 macrophages.

Calendula herba concentration-dependently inhibited NF-κB activation (IC₅₀: 74.9 µg/ml). In a cellfree enzyme inhibition assay, Calendula herba concentration-dependently inhibited both, 5-LOX and COX-2 enzyme activity (IC₅₀: 39.6 µg/ml and 29.4 µg/ml). In addition, Calendula herba strongly induced the release of lipid mediators, such as SPM (specialized pro-resolving mediators), 15-LOX products and PUFAs (poly unsaturated fatty acids) in human M2 macrophages at a concentration of 100 µg/ml.

These data underline the potent inhibition of inflammatory and pain-related mediators and indicate the pro-resolving capacity of the Weleda Calendula herba extract (Fig. 1). The described mechanisms of action of Calendula herba contribute to the therapeutic efficacy of Calendula preparations.

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P300 The unique combination of *Malva sylvestris* L. flower extract and hyaluronic acid provides advantageous efficacy for treatment of dry eye disease

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Dry eye disease (DED) is a multifactorial disease with symptoms such as itching, foreign body sensation, redness and pain [1]. The hyperosmolar environment due to loss of tear volume triggers the release of pro-inflammatory mediators and oxidative stress due to the release of oxygen radicals [2]. First line treatment for mild to severe DED is mostly based on eye drops containing hyaluronic acid (HA) [3]. However, treatment with HA alone neglects oxidative stress.

The herbal *Malva sylvestris* L. flos (MS) extract was investigated in the oxygen radical absorbance capacity (ORAC) assay and the cellular antioxidant assay (CAA). Physicochemical studies with MS and HA were performed to determine surface tension, coefficient of friction and mucoadhesion. An experimental *in vitro* 3D model of DED using human reconstructed corneal epithelium (HCE) was used to analyse the therapeutic capacity of MS and HA.

In both cell-free and cell-based antioxidant assays, MS showed oxygen radical scavenging activity, indicating its antioxidant capacity and cellular uptake. Using HA-containing eye drops plus MS extract, surface tension was significantly reduced compared to a HA-only preparation. Notably, the combination of HA and MS significantly reduced the coefficient of friction compared to single compounds. Additionally, the MS mucilage and HA demonstrated significant mucoadhesion. In the 3D HCE model for dry eye associated ocular surface damage MS and HA showed potent tissue protective effects.

These data indicate that the MS flower extract exerts an effective contribution to common HA treatments in DED therapy due to its comprehensive beneficial antioxidant and physicochemical properties.

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P301 Evaluation of an herbal preparation for treatment of digestive disorders regarding growth of selected intestinal bacteria in single culture and co-culture tests

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The herbal preparation Amara-Drops is a traditional remedy for treatment of digestive disorders like fullness, heartburn, nausea and gastrointestinal dysmotility. It is composed of nine hydroethanolic herbal extracts, *Artemisia absinthium* (AA), *Centaureum erythraea* (CE), *Cichorium intybus* (CI), *Gentiana lutea* (GL), *Juniperus communis* (JC), *Achillea millefolium* (AM), *Peucedanum ostruthium* (PO), *Salvia officinalis* (SO) and *Taraxacum* (T). A well functioning digestion is inevitably linked to a healthy intestinal microbiota [1, 2]. Thus, medication should not significantly interfere with intestinal commensal microbes. We examined the interaction of the herbal extracts with *Lactiplantibacillus plantarum*, *Ligilactobacillus salivarius*, *Escherichia coli*, *Enterobacter cloacae* and *Bacillus subtilis* using single culture and co-culture tests *in vitro*. Cloves extract or antibiotic tetracycline were used as reference compounds. Bacteria were incubated in liquid culture under constant shaking at 37 °C for up to 7 h together with test samples or controls. With exception of *B. subtilis*, the growth of tested bacteria was not inhibited at concentrations < 5 mg/ml. At very high concentrations between 5–10 mg/ml only JC and SO showed antibacterial effects towards *L. plantarum* and *L. salivarius*, as well as GL, PO, SO and T towards *E. cloacae*, respectively. The reference cloves extract and antibiotic completely inhibited the growth of tested bacterial strains. In the experimental co-culture test growth of *L. plantarum* was only impaired at the highest test concentration of 10 mg/ml, while growth of *E. coli* was not affected. Overall, these results indicate that Amara-Drops are compatible with the intestinal commensal bacterial strains and might be considered as microbiome-friendly.

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P302 Bioguided optimization of α -glucosidase inhibitors by ultrasound-assisted extraction

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Gunnera tinctoria (Molina) Mirbel is a perennial plant native to South America, widely recognized for its ecological adaptability and traditional medicinal uses [1]. While the genus *Gunnera* has been investigated for its bioactive properties, comprehensive studies on the phytochemical composition and pharmacological potential of *G. tinctoria* remain scarce. Previous reports suggest the presence of phenolic compounds and other secondary metabolites with antioxidant and enzyme-inhibitory activities [2]. Our research group has recently identified that rhizome extracts of *G. tinctoria* exhibit significant glucosidase inhibitory activity, highlighting its potential for applications in metabolic disorders. However, the bioactive molecules responsible for this effect have not yet been isolated and characterized. Additionally, *G. tinctoria* is an invasive species in the Azores, where it threatens local ecosystems [3]. Thus, exploring its bioactive potential could promote the valorization of this abundant biomass while mitigating its environmental impact. This study optimized the ultrasound-assisted extraction (UAE) of *G. tinctoria* rhizomes to improve the yield and selectivity of bioactive compounds. UAE parameters—including solvent selection, extraction time, and ultrasound amplitude—were systematically evaluated to maximize extraction efficiency while preserving bioactivity. Furthermore, the functionalization of magnetic particles with α -glucosidase was also studied and optimized, with the goal of obtaining an affinity-based matrix for the separation of the more active extracts (Fig. 1). This approach integrates advanced extraction techniques with affinity-based screening to accelerate the discovery of natural enzyme inhibitors. The findings from this study are expected to contribute to the sustainable exploitation of *G. tinctoria* as a source of bioactive compounds with potential applications in medicinal formulations.

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P303 *Opuntia-ficus-indica* extract enhances oxygen consumption and glycogen metabolism in skeletal muscle cells

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Opuntia species have been well documented for their benefits in human health, particularly blood glucose management. Recovera™, a proprietary extract developed by Finzelberg, combines the cladodes and fruit skins of *Opuntia-ficus-indica* (OFI) and is characterized by a high content of soluble fibers as well

piscidic acid as key single substance. Based on internal research and supported by several clinical studies, Recovera™ has demonstrated potent anti-hyperglycaemic and antidiabetic properties [1]. This *in-vitro* study aimed to elucidate the mechanisms of action of OFI and piscidic acid, with a particular focus on its potential to enhance muscle recovery following exercise. To this end, we evaluated glycogen content and oxygen consumption under both basal and exercise-mimicking conditions. The C2C12 mouse myoblast cell line was selected due to its ability to differentiate into contractile myotubes expressing key muscle-specific proteins. Insulin served as a positive control for glycogen synthesis, while caffeine was employed to simulate a hypercontractile state [2]. After the study of different biomarkers related with muscle metabolism, we can conclude: in exercise-like conditions (caffeine pretreatment) we observe that, after treatment with extract or piscidic acid, cells return to basal conditions (control); while oxygen consumption shows significant differences, this could indicate the return to physiological activity of the mitochondria. An increase in basal conditions after treatments it might be related with a proliferative effect. Results suggest that OFI extract preparation Recovera™ and piscidic acid can counteract the negative effect of caffeine (exercise-like conditions), helping muscle function restoration and relaxation in an *in vitro* model.

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P304 Potential of *Xylopia aethiopica* as Alternative Therapeutics against *Staphylococcus aureus*

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Xylopia aethiopica is a fragrant evergreen tree belong to Annonaceae family with an average height of 20 m. It is a native to the 'Sahel' savanna zones of Africa, occurring from Senegal to Sudan [1, 2]. The dried fruits are used in herbal medicine to treat cough, stomach disorder, infections, malaria, syphilis and dysentery [3]. This study aimed to evaluate the antimicrobial potential of fruit extracts against *Staphylococcus aureus*, and to identify the chemical components in the active extracts. The decoction, dichloromethane, ethyl acetate and hexane extracts effectively inhibited the growth of *S. aureus* showing inhibition zone diameters from 23.8–27.2 mm. The methanol extract displayed a MIC value at 313 µg/ml, whereas the ethyl acetate and dichloromethane extracts gave a MIC value of 625 µg/ml. The chemical analysis using UPLC/QTOF-MS revealed the presence of wide range of different chemical classes in the ethyl acetate extracts, including phenols as chlorogenic acid (m/z 354.3090), rutin (m/z 610.5180), and two hydroxycinnamic acid of coumaroylquinic acid at m/z 338.31 and caffeoylquinic acid (Neochlorogenic Acid) at m/z 354.3. In addition, two kauranes class diterpene such as xylopic acid (m/z 360.4871) and kaurenoic acid (m/z 302.451) were detected. This study provided additional scientific evidence for the use of *Xylopia* extracts-based therapeutics to treat bacterial infections in African traditional medicine. Besides, our results demonstrate that *X. aethiopica* extracts could be used for future opportunities to develop sustainable, plant-based antimicrobial therapies. Future research should focus on identifying and optimizing the bioactive compounds in extracts and on isolating the bioactive compounds for clinical and pharmaceutical applications.

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P306 Correlation between the biological activities and chemical profile of extracts from *Cistus incanus*

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Some herbs have potential antidiabetic effects by reducing oxidative stress and inhibiting enzymes that break down complex sugars, which helps reduce hyperglycemia. In this study chemical composition, antioxidant potential and α -amylase inhibition by the aqueous and ethanolic extracts from *Cistus incanus* are presented. *Cistus incanus* belongs to the *Cistaceae* family. It has been used in folk medicine for centuries. For this reason it is subject of interest for researchers looking for new compounds of plant origin with high biological activity. Antioxidant activity of aqueous and ethanolic extracts was evaluated in terms of free radical scavenging (DPPH, ABTS), reducing power (CUPRAC, FRAP), total phenolic content (TPC), and possibility to iron(II) ion chelation. Enzyme inhibitory potential were tested against α -amylase using 3,5- dinitrosalicylic acid method. It was found that ethanol extracts of cistus were characterized by higher antioxidant activity and also inhibited alpha amylase activity to a greater extent than aqueous extracts. Determination of the concentration of polyphenolic compounds (GC-MS) and selected elements (ICP-MS) in the test samples was also done. *Cistus incanus* is a source of phenolic compounds with antioxidant and antidiabetic effects. It came out that gallic and ellagic acids are the most represented in studied samples. The presence of phenylacetic, benzoic and cinnamic acid derivatives as well as flavonoids such as quercetin and kaempferol was also demonstrated. A correlation between the biological activity of cistus extracts and the content of phenolic compounds was found. The results of the studies indicate a high antioxidant potential of ethanol extracts from cistus and its clear health-promoting potential.

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P307 Sumac (*Rhus coriaria* L.) as a source of bioactive compounds: Unlocking its nutraceutical potential

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Sumac, a tangy, deep-red spice derived from the dried and ground drupes of *Rhus coriaria* L. (Anacardiaceae), is used in Middle Eastern diet. Beyond its culinary appeal, sumac fruits are rich in organic acids, flavonoids, and tannins, phytochemicals renowned for their potent antioxidant properties and health-promoting potential [1, 2]. This study evaluated the nutraceutical potential of a phenolic-rich extract obtained from sumac drupes. The extract was chemically characterized using HILICxRP-LCPDA-ESI/MS. Its antioxidant activity

was measured through DPPH and ABTS assays, while its inhibitory effect on α -amylase and α -glucosidase—key enzymes in glucose metabolism—was assessed. The extract's ability to counteract oxidative stress was further tested in HepG2 human liver cells exposed to hydrogen peroxide. Phytochemical analysis revealed that gallic acid and its derivatives are the major phenolic constituents (2,215 mg/100 g), making up 89% of the total bioactive compounds. The extract exhibited strong antioxidant activity (DPPH: 1.02 mmol TE/g; ABTS: 1.76 mmol TE/g) and effectively inhibited α -amylase (IC₅₀: 2.28 mg/mL) and α -glucosidase (IC₅₀: 18.5 μ g/mL), with the latter surpassing the pharmaceutical inhibitor acarbose in potency. Moreover, it significantly reduced reactive oxygen species in HepG2 cells under oxidative stress. These findings highlight sumac as a valuable source of bioactive phenolics with promising applications in nutraceutical and pharmaceutical formulations. Further studies are needed to clarify its mechanisms of action.

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P308 Microbiota-mediated biotransformation of triterpene glycosides from *Cimicifuga racemosa* extract Ze 450

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The pharmacological efficacy of *Cimicifuga racemosa* extract Ze 450 in treating menopausal symptoms is thought to be linked to triterpene glycosides (TTGs) [1, 2]. Emerging evidence suggests that intestinal microbiota may metabolize TTGs into bioactive aglycones, potentially contributing to the extract's therapeutic effects [3]. This study investigated the biotransformation of selected TTGs (23-epi-26-deoxyactein, actein, cimigenol-3- β -D-xyloside, and cimigenol-3- α -L-arabinoside) using *ex vivo* incubation with human stool samples (n=8) under strict anaerobic conditions for 48 hours [4]. Qualitative analysis was performed using UHPLC-qTOF-MS, revealing complete degradation of all investigated TTGs after incubation. Quantitative analysis of the biotransformation of deoxyactein showed a significant increase in its aglycone, which varied among donors, indicating individual differences in microbial activity and metabolic capacity. Stability of deoxyactein over gastrointestinal phases was proven by using simulated fluids across the oral and gastric phases. These findings confirm a microbiota-mediated cleavage of the glycosidic moieties, yielding potentially active aglycones. Effects of TTGs and Ze 450 on microbial community were studied using alpha and beta diversity metrics, along with modulation of short-chain fatty acids (SCFAs). No significant adverse effects on microbial community structure or SCFA profiles were observed, though minor shifts in taxonomic profiles (Bray-Curtis distance) were noted for select donors. This proof-of-concept study highlights the importance of individual microbiome variability in TTG metabolism and lays the foundation for personalized phytotherapeutic strategies. Ongoing work aims to correlate microbiome taxonomy with metabolic conversion efficiency to enhance therapeutic consistency and optimize the clinical efficacy of Ze 450.

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P309 Chemodiversity triggering via coculture of endophytic fungi strains of *Humicola fuscoatra* and *Nemania bipapillata* isolated from marine red algae

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The marine ecosystem has emerged as a promising, yet underexplored, source of natural products due to its rich biodiversity and chemodiversity. Within this domain, endophytic fungi associated with marine algae have shown great potential for providing value-added metabolites and bioprospection [1]. However, most genes related to biosynthesis in microorganisms remain silenced under standard laboratory growth conditions. Some approaches have been used to trigger activation of silenced biosynthetic pathways such as coculture, which consists of inoculating two or more microorganism strains together, allowing them to induce physiological and chemical stimuli [2]. In this study, two fungal strains isolated from the red alga *Asparagopsis taxiformis* were identified as *Humicola fuscoatra* (At02) and *Nemania bipapillata* (At05), were grown in Petri dishes containing PDA culture medium and incubated for 10 days at 25° C. The two strains were inoculated both separately and together in Erlenmeyer flasks containing ultrapure water and malt growth medium in static mode for 21 days. After filtration of the fermented broths from the mycelium, the filtrate was partitioned with EtOAc and evaporated to yield the extracts. The control extracts (monoculture) and the coculture extract were analyzed by UPLC-MS/MS and the data submitted to GNPS molecular networking platform [3], which provided annotation of constituents produced only in coculture as isoresperin (1), gentiopicroside (2), and a cluster with two diterpenes, kaurenoic acid (3) and 17-hydroxy-15,16-epoxykauran-18-oic acid (4) and the sesquiterpenoid verrucarol (5). Such results contribute to the understanding of the chemodiversity of marine endophytic fungi, opening perspectives for the sustainable exploration of this biome, in alignment with the UN-SDGs.

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P310 Phytochemical and pharmacological investigation of oxidized secoiridoids from *Ligustrum lucidum*

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In the pursuit of alternative plant sources rich in the secoiridoid glycosides oleuropein and ligstroside, our research focused on *Ligustrum lucidum* (Oleaceae) [1]. Preliminary analysis of its leaves led to the isolation of two novel oxidized analogues, oleuropeinic and ligstrosidic acid. These compounds were further investigated due to their significant pharmacological interest. The study aimed to comparatively evaluate various parts of *L. lucidum*, *Fraxinus angustifolia*, and *Ligustrum vulgare*, all Oleaceae family species, focusing on the content of the two acids and their overall metabolic profiles using HPTLC, HPLC-DAD, and UHPLC-HRMS/MS. Seasonal variation and drying methods of the plant material were also evaluated. The acids were efficiently isolated through a refined workflow incorporating CPC and semi-preparative HPLC. Their structures were confirmed by NMR and UHPLC-HRMS/MS, and optical rotation values were determined [2]. In total, 141 secondary metabolites were identified across all extracts. Comparative phytochemical analyses revealed notable variations in acid content based on species, plant part, and extraction conditions. To explore potential biological response, hepatoprotective activity was evaluated for ligstrosidic, oleuropein, ligstrosidic and oleuropeinic acid, respectively [3]. Notably, ligstrosidic acid demonstrated remarkable hepatoprotective effects compared to the positive control, oleuropein, suggesting the oxidized analogues may possess enhanced biological activity. These findings not only establish *L. lucidum* as a promising source of bioactive oxidized secoiridoids but also highlight the importance of extraction parameters and species selection in optimizing yield and bioactivity of plant-derived metabolites.

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P311 8-Shogaol, a natural compound derived from *Zingiber officinale*, ameliorates rheumatoid arthritis by targeting TAK1

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Rheumatoid arthritis (RA) is a chronic autoimmune disease marked by inflammation of the synovial membrane and progressive joint damage [1]. Without proper treatment, it can lead to permanent joint destruction and reduced life expectancy. Despite advancements in understanding RA pathogenesis and the development of new therapies, many patients still fail to achieve remission and

continue to suffer from functional decline [2]. Fibroblast-like synoviocytes (FLS), which play a central role in RA progression, have emerged as a promising therapeutic target [3]. To discover compounds that can effectively suppress FLS-driven inflammation, 315 natural extracts were screened for their ability to inhibit IL-17-induced IL-6 production. Among these, *Zingiber officinale* showed the most promising results. Further investigation identified 8-shogaol as the active compound responsible for this effect. 8-Shogaol significantly reduced inflammation and cell migration triggered by TNF- α , IL-1 β , and IL-17 in FLS derived from RA patients as well as in a 3D synovial culture model. Mechanistically, 8-shogaol directly inhibited TAK1, leading to suppression of downstream IKK, Akt, and MAPK signaling pathways. In vivo, treatment with 8-shogaol reduced paw swelling, improved gait, and restored joint structure in adjuvant-induced arthritis (AIA) rats, while also lowering inflammatory markers in the affected joints. Overall, this study identifies 8-shogaol as a novel anti-RA agent that targets TAK1 to alleviate synovial inflammation and joint pathology.

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P312 Effect of methyl jasmonate, salicylic acid and yeast extract on secondary metabolites production in *Arnica montana* tissue cultures

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Arnica montana L. is a valuable but relatively unexplored medicinal plant belonging to the Asteraceae family. Although the properties of secondary metabolites found in arnica extracts have been known for centuries (e.g. their anti-inflammatory, antioxidant, antibacterial, or antifungal activity) [1,2], little is known on the molecular background of their biosynthesis. Due to the high pharmaceutical value of this species and its endangered status, the aim of our research was the enhancement of secondary metabolites biosynthesis in *A. montana* growing in tissue cultures through elicitation. The influence of three types of elicitors (methyl jasmonate, salicylic acid, and yeast extract) at three concentrations each was tested. The impact of elicitation on micropropagation, growth parameters and accumulation of secondary metabolites were assessed. Moreover, the expression of selected genes of interest involved in secondary metabolites biosynthesis was evaluated. The target analyzed genes encoded key enzymes involved in the phenylpropanoid pathway, the mevalonate pathway (MVA), the non-mevalonate pathway (MEP/DOXP), and sesquiterpene lactones biosynthesis. The obtained results shed light on the regulation of secondary metabolism in *Arnica* in response to different elicitors agent and provide the basis for current and future molecular studies in this important medicinal species.

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P313 Total hydroxycinnamic acid quantification and micromorphological characterization of balkan and imported herbal substances from Lamiaceae subfamilies

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Herbal substances from Lamiaceae species have both traditional and contemporary phytotherapeutic applications and are a potent source of bioactive compounds. Phenolic derivatives, such as rosmarinic and chlorogenic acid, exhibit multiple activities, and rosmarinic acid has recently been shown to play important roles in wound healing [1,2]. Few comprehensive comparative hydroxycinnamic acid assays have been conducted on genera from different Lamiaceae subfamilies, and none have been performed for herbal substances originating from or imported to the Balkans. Our primary goal is to elucidate the total hydroxycinnamic acid contents of herbal substances from Ajugoideae, Lamioideae, and Nepetoideae taxa by performing a spectrophotometric determination as described in the European Pharmacopoeia [3]. A secondary objective is to describe the micromorphological characteristics in bright field of herbal substances that do not have established individual Pharmacopoeial monographs. We report the total hydroxycinnamic acid contents expressed as rosmarinic and chlorogenic acid of 20 herbal substances from 19 taxa and the micromorphological characteristics of 12 herbal substances. While herbal substances from Nepetoideae species accumulate the highest contents of phenolic acid derivatives, the dried aerial parts of Ajugoideae species, such as *Teucrium polium* L., also accumulate relatively significant contents of hydroxycinnamic acids (5.41 $\pm$ 0.4 %).

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P314 Natural deep eutectic solvents (NADES) for the extraction of bioactive compounds from quinoa (*Chenopodium quinoa* Willd.) leaves

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Natural deep eutectic solvents (NADES) have emerged as a promising, eco-friendly alternative to petrochemicals to extract plant metabolites. As the demand for sustainable, “green” ingredients for industrial applications is growing, those solvents are purported to develop extracts with interesting phyto-

chemical fingerprints and biological activities. Given the interest of flavonoids from *Chenopodium quinoa* Willd. leaves, an efficient “green” extraction method was developed by investigating eight NADES with defined molar ratios, i.e. malic acid-choline chloride (chcl)-water (w) (1:1:2, N1), chcl-glucose-w (5:2:5, N2), proline-malic acid-w (1:1:3, N3), glucose-fructose-sucrose-w (1:1:1:11, N4), 1,2-propanediol-chcl-w (1:1:1, N5), lactic acid-glucose-w (5:1:3, N6), glycerol-chcl-w (2:1:1, N7) and xylitol-chcl-w (1:2:3, N8). Rheological measurements of all NADES confirmed their pseudoplastic behaviors. To improve the extraction processes through lowering NADES viscosity without disturbance of eutectic properties, differential scanning calorimetry (DSC) was applied to determine the maximum amount of water that could be added to NADES (N1, N2, N3 and N4; 17.5 %, 20 %, 10 % and 10 % w/w, respectively). The phytochemical compositions of NADES extracts were analyzed by high-performance thin-layer chromatography (HPTLC) and their free radical scavenging and α -amylase inhibitory properties were assessed by HPTLC-bioautography. N2, diluted with 20 % of water, and N7 presented the best potential for replacing methanol for an eco-friendly extraction of flavonoids, radical scavengers, and α -amylase inhibitors from quinoa leaves. Their biological properties, combined with a good understanding of both thermal behavior and viscosity, make the obtained quinoa leaves NADES extracts good candidates for direct incorporation in nutraceutical formulations.

P315 Terpenoids with selective cytotoxic activity against HCC70 breast cancer cell line from *Leonotis ocyimifolia*

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Triple Negative Breast Cancer (TNBC) is a primary global health concern, especially in sub-Saharan Africa, where treatment options are limited. Medicinal plants, such as those from the genus *Leonotis*, offer promising therapeutic agents due to their anticancer and anti-inflammatory properties. This study aimed to isolate and characterize labdane diterpenoids and triterpenoids from *Leonotis ocyimifolia* and to evaluate efficacy against breast cancer cells. Compounds from *Leonotis ocyimifolia* were characterised using spectroscopic data such as IR, 1D and 2D NMR, and MS spectrometry and evaluated for cytotoxic activity against triple negative breast cancer (HCC70), hormone receptor-positive breast cancer (MCF-7), and non-tumorigenic mammary epithelial cell lines (MCF-12A). The structural elucidation processes afforded eleven compounds, including two labdane diterpenoids (13S-predubiin and 13S-nepetaefolin) and one unreported tetracyclic triterpenoid (3,10-epoxy-9-epi-cucurbita-24-ene). The isolated labdane diterpenoids exhibited selective activity against HCC70 cells, with 13S-nepetaefolin displaying the most potent cytotoxicity (IC₅₀ of 24.65 μ M), while its isomer, nepetaefolin, showed no significant activity (IC₅₀ > 200 μ M) [1–3].

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P316 Dibenzoylmethane from *Hottonia palustris* and its analogs serves as a novel β -diketone pore blockers of large-conductance calcium-activated potassium channel

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Large-conductance calcium-activated potassium (BKCa) channels are broadly expressed in mammalian tissues and organelles, modulating membrane excitability, calcium signaling, and cell survival. These channels are known for their multimodal regulation and sensitivity to numerous endogenous and exogenous small molecules [1]. Although BKCa channels are involved in several pathological processes, no clinically approved drugs selectively targeting them are currently available. Our study identified dibenzoylmethane (DBM), a natural compound derived from *Hottonia palustris* (Primulaceae), as a BKCa channel inhibitor [2]. DBM reduced channel open probability in a dose-dependent manner and significantly decreased the mean open time, inducing a flickering gating pattern characteristic of pore blockade. Functional competition with the high-affinity pore blocker paxilline and molecular dynamics simulations demonstrating DBM-induced dehydration of the central cavity support a pore-binding mechanism. To investigate the structural determinants of DBM's activity, we also tested three analogs: phenyl-1,3-butanedione (PBD), *trans*-chalcone (T-Ch), and (E)-1,3-diphenylprop-2-en-1-ol (DPE). Both T-Ch and DPE exhibited inhibitory profiles similar to DBM, while PBD was less effective and required higher concentrations to achieve inhibition. These results suggest that the diphenyl substitution in these compounds contributes to the inhibition of BKCa channels.

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P317 Identification cytotoxicity and wound healing effects of various extracts of *Sanguisorba minor* Scop. seeds

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The genus *Sanguisorba* comprises species within the Rosaceae family, indigenous to Asia, Western Europe, and North America [1]. Plants of the *Sanguisorba* genus, originating from the Latin terms “sanguis” (blood) and “sorbere” (to suck), which signifies the cessation of bleeding, have traditionally been utilised for this purpose [2]. This study examined the cytotoxicity and wound healing effects of extracts with varying polarity derived from seeds of the *Sanguisorba minor* Scop. Fractional extracts of chloroform, ethanol (96 %), and distilled wa-

ter were obtained from *S. minor* seeds via a Soxhlet apparatus. The phenolic compounds included in the resultant extracts were identified using LC-MS/MS analysis of 32 distinct reference compounds. According to the analysis results, the extracts carry 19 different compounds, including vanillin and gallic acid as main compounds in different proportions. The anticancer effects of the extracts on SH-SY human neuroblastoma cells were examined using the MTT test. The water extract exhibited cytotoxic activity with an IC₅₀ value of 214.3 µg/mL; however, no significant results were seen for the chloroform and ethanol extracts. The therapeutic effects of all extracts on *in vitro* wound healing in L929 cell lines were examined. While the chloroform extract killed the cells in a short time, the water extract showed a rapid wound closure compared to the control group. This research is the initial investigation into the wound healing properties of the *S. minor* and constitutes the first unique study on the cytotoxicity and wound healing effects of its seed extracts. Furthermore, additional *in vitro* and *in vivo* investigations are required for wound healing and cytotoxicity.

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P318 Plant extract-enriched hydrogels: Exploring the therapeutic potential, biocompatibility and antioxidant activity of propolis, Echinacea, and turmeric for skin regeneration

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Hydrogels, particularly those derived from natural polymers, serve as effective delivery systems for active compounds, improving stability, bioavailability, and therapeutic outcomes in wound healing and skin regeneration. The use of antioxidant substances such as propolis, Echinacea, or turmeric as ingredients enriching hydrogel dressings is increasingly being considered [1–3]. Propolis is characterized by its flavonoid content and caffeic acid derivatives, Echinacea for alkylamides and polysaccharides, while Turmeric is primarily recognized for curcumin, all contributing to their antioxidant potential. This research aimed to develop a hydrogel dressing enriched with skin-regenerating substances and antioxidant plant extracts. Hydrogels based on polyvinylpyrrolidone and gellan gum, enriched with allantoin (0.5%) and D-panthenol (0.5%), and 1% extracts of propolis, echinacea, and turmeric, were investigated. Cytotoxicity, assessed using the MTT assay in accordance with ISO 10993-12:2012, revealed high biocompatibility for the hydrogel with 1% propolis, while echinacea and turmeric showed slightly lower biocompatibility but maintained viability above 70%. Antioxidant activity was measured using the ABTS method, assessing the ability to scavenge the ABTS radical cation, reduced by antioxidants. The resulting color intensity was measured using UV-Vis spectroscopy at 734 nm. The concentration of antioxidant substances released from the hydrogel was determined at 2, 4, 6, 8, and 24 hours. The results showed a gradual release of antioxidants, with the hydrogel's antioxidant effect increasing within 8 hours, followed by stabilization. The tested hydrogels demonstrated high biocompatibility and antioxidant activity, especially with 1% propolis extract, enhancing their potential for wound healing and skin regeneration.

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P319 Exploring the anti-inflammatory potential of CBDA

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Cannabidiolic acid (CBDA) is a poorly understood natural molecule derived from (*Cannabis sativa* L.). Its potential health benefits have long been overlooked due to the greater scientific focus on its neutral derivative, cannabidiol (CBD) [1]. Although its pharmacological and nutraceutical enforceability is still a long way off, the significant concentration of this molecule in food-grown hemp cultivars indicates that more research is needed to completely understand its chemical and biological characteristics as well as to fully and well exploit it, especially because its content is massive in hemp wastes [2]. This study investigates the anti-inflammatory effects of CBDA, focusing on its molecular mechanisms through the modulation of pro-inflammatory cytokines and the regulation of inflammatory signaling pathways in an *in vitro* cellular model. Given the strong interplay between the tumor and immune microenvironment, experiments were conducted on THP-1 cells differentiated into macrophages and HL-60 cells differentiated into neutrophils, as these immune cells play a pivotal role in inflammation. To explore the link between inflammation and cancer, we evaluated (i) the release of specific pro-inflammatory and anti-inflammatory mediators and (ii) the inhibition of both nitric oxide synthesis and intracellular levels of reactive oxygen species. The findings of this study provide new insights into the anti-inflammatory properties of CBDA, highlighting its ability to modulate key inflammatory markers. However, further research is needed to fully elucidate its therapeutic potential and underlying mechanisms of action.

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P320 YaBiNaPA's contribution to the sustainably management of *Vernonia senso lato* species in Cameroon

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In Cameroon, *Vernonia senso lato* species is a national treasure. The popularity of the genus comes from “Ndole”, a succulent dish made from the leaves of *V. amygdalina* and *V. calvoana*. The leaves also constitute the first kit the popula-

tion used against malaria. Other *Vernonia* species are also used traditionally across the country like the roots of *V. kotschyana*, used to relieve gastric pain, or the rhizomes of *V. guineensis*, used to cure prostate cancer. Besides, many Cameroonian species remain unknown, despite extensive reports and recent reclassification within *Vernonia s.l.* The present study overlooked the chemistry and biology of Cameroonian *Vernonia s.l.* species for a better management of their traditional uses.

Results gathered to date support the proposal of a *Vernonia s.l.* species cluster into three main groups depending on their chemistry. They are either sesquiterpene lactone, sitostane-type steroids and aliphatic acetylene producers. Moreover, isolated compounds shown significant potency against microbial strains and cancer cell lines. Also, preliminary results of *in silico* studies suggest promising binding mode in the allosteric pocket of the human 5-LOX enzyme, one of the three therapeutic targets for the anti-inflammatory activity docked, of some of the steroid derivatives isolated from *V. kotschyana*, partly due to both OH and C=O in the kotschyanosides. These results are still to be confirmed with *in vitro* studies. Outcomes from this study will provide solid ground to inform the population on possible benefits of more *Vernonia s.l.* species as well as scientific background of the species currently in use traditionally.

P321 Effects of myristoleic acid on the inhibition of 1-nitropyrene-induced apoptosis in dermal papilla cells

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Diesel exhaust is a major source of particulate matter and gaseous pollutants [1]. 1-Nitropyrene (1-NP), a key nitro-polycyclic aromatic hydrocarbon in diesel particulate matter, is a known mutagen and carcinogen [1], but its effect on hair loss unclear. Myristoleic acid (MA) is an omega-5 monounsaturated fatty acid obtained from the seeds of plant from the Myristicaceae family [2]. MA was reported to enhance Wnt reporter activity in dermal papilla cells (DPCs) [2], but it has not been studied the effect of MA on DPCs under 1-NP exposure. In this study, we investigated the effects of 1-NP on hair growth signals in DPCs, the central regulators of the hair cycle, and its impact on MA-pretreated DPCs. When DPCs treated with 1-NP, the proliferation of DPCs significantly decreased in a concentration-dependent manner. 1-NP induced apoptosis by increasing Bax/Bcl-2, cleaved caspase-3, and poly (ADP-ribose) polymerase levels. Furthermore, 1-NP decreased the mRNA levels of the Wnt gene family, potentially impairing Wnt/ β -catenin pathway that regulates the hair cycle. However, when 1-NP was applied to MA-pretreated DPCs, DPCs proliferation increased compared to control (DPCs exposed exclusively to 1-NP). These results suggest that 1-NP suppresses hair growth signals by inducing apoptosis and disrupting Wnt/ β -catenin pathway in DPCs, whereas MA mitigates cytotoxic effects of 1-NP.

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P322 Phytochemical insights and bioherbicidal applications of *Cynara cardunculus* L.

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Cynara cardunculus L. demonstrates notable phytotoxic effects against various weed species, largely attributed to its rich profile of bioactive secondary metabolites. This study aims to explore the relationship between the presence and concentration of these compounds and their herbicidal activity. A comprehensive review of existing literature on the aerial parts of *C. cardunculus* L. reveals a diverse spectrum of allelopathic constituents, including flavonoids and their glucosides, triterpenes, polyphenols, sesquiterpene lactones. Among the most studied compounds, sesquiterpene lactones such as cynaropicrin and gro-sheimin, as well as flavonoids like luteolin and apigenin, stand out as key contributors to the plant's phytotoxicity. Compounds such as aguerin B, *p*-coumaric acid, and quercetin further enhance its allelopathic potential. The phytochemical investigation combined a bibliographic analysis with experimental validation through TLC and UHPLC-MS across three *C. cardunculus* L. genotypes. Extracts were obtained using conventional solvents, with ethyl acetate yielding the most promising results. This extract exhibited high herbicidal activity and a rich composition of bioactive compounds, including cynaropicrin, chlorogenic acid, luteolin, cynaroside, apigenin, aguerin B, cynarinin A/B, and *p*-coumaroylquinic acid. The wild genotype displayed the highest metabolite concentrations. These findings suggest a strong correlation between the herbicidal potential of *C. cardunculus* and the concentration of specific compounds. Future work will focus on optimizing extraction techniques, identifying additional active metabolites, and testing compound synergies under pre-emergence conditions. This approach supports the development of *C. cardunculus*-based bioherbicides for sustainable weed management.

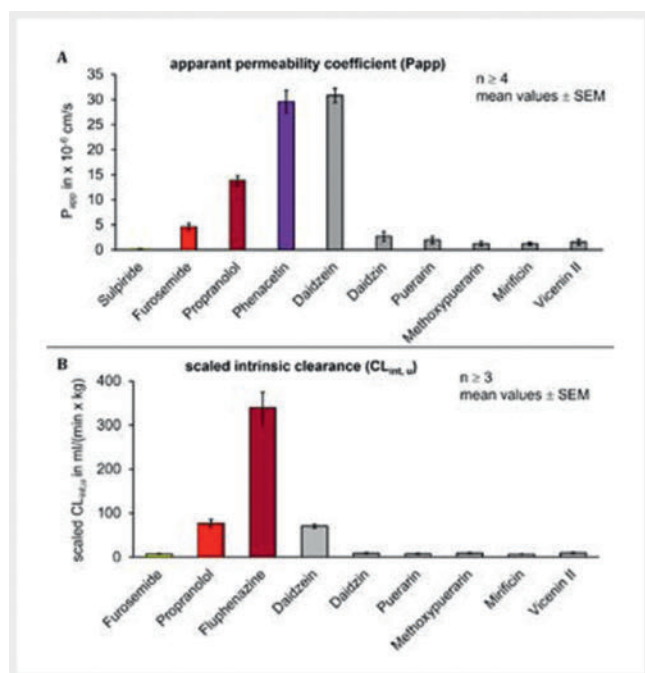
P323 *In vitro* absorption and metabolism of isoflavone C-glycosides, O-glycosides, and their aglycons

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Isoflavones are a class of phytochemicals predominantly found in members of the Fabaceae family, particularly in leguminous plants such as soybeans, clover, and beans [1]. Their potential health-promoting effects have garnered considerable scientific interest in recent years, especially regarding their role as alternative therapeutic agents in the prevention and management of cancer, cardiovascular diseases, and menopausal disorders [2]. The extent of isoflavones' biological activity in humans is critically influenced by their absorption and metabolic transformation, primarily occurring in the small intestine and liver. Accordingly, this project aimed to establish an *in vitro* model capable of predicting the oral bioavailability of isoflavones with special emphasis on their C-glycosides. Based on the absorption in a Caco-2 cell model, the permeability coefficients of the compounds were determined (► Fig. 1A). In addition, the scaled intrinsic clearance rates were calculated based on the absorption of the compounds in the HepaRG™ cell model (► Fig. 1B). By calibrating the experiments with well-investigated model substances (sulpiride, furosemide, propranolol, phenacetin, and fluphenazine), these results could be correlated to *in vivo* intestinal absorption and hepatic clearance. Moreover, numerous intestinal and hepatic metabolites were detected using UHPLC-DAD-MS. The formation of metabolites was verified using control substances with known *in vivo* metabolites. Thereby, a wide range of phase I and phase II metabolites was identified for the isoflavones. In addition, the associated aglycons could also be

detected as metabolites for the C-glycosides, which indicates that both Caco-2 and HepaRG™ cells can cleave C-C bonds.



► **Fig. 1** Apparent permeability coefficients and scaled intrinsic clearance rates of isoflavones compared to control compounds.

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P324 Cosmeceutical activity of *Myrcia neoobscura* hydroalcoholic extract and its fractions

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Myrcia neoobscura E.Lucas & C.E.Wilson, Myrtaceae is a subshrubby perennial species endemic to Brazil where it grows in the Northeast, Southeast, and South regions. In this study, cosmeceutical activity of several fractions of hydroalcoholic (70 %) *M. neoobscura* extract was investigated. For preparation of samples, the plant material was twice macerated in ethanol 70 % each time for seven days. The extracts were combined, filtered and evaporated. The obtained hydroalcoholic crude extract (CHE) was resuspended in 20 % ethanol, stored at 4 °C for 12 h and filtered to yield insoluble precipitate (IF) and the filtrate. The latter was partitioned in solvents of increasing polarity (hexane > dichloromethane > ethyl acetate), yielding hexane, dichloromethane (discarded), ethyl acetate (EAF) and aqueous fraction (AF). Cosmeceutical activity of the crude extract and the fractions was investigated as the inhibitory activity on elastase, collagenase, lipoxygenase, and tyrosinase. Results were expressed as the respective IC₅₀ values. The extracts demonstrated the activity in all the performed assays. The most active tyrosinase, collagenase, and lipoxygenase inhibitors

were IF, EAF and CHE, respectively. While in most cases the activity was lower than the activity of the respective standard inhibitors, CHE (318.0 µg/mL ± 2.6 µg/mL) and AF (315.2 µg/mL ± 4.3 µg/mL) statistically reached the activity of ursolic acid (313.6 µg/mL ± 38.2 µg/mL) in the anti-elastase assay. Additional studies aiming at isolation of the compound(s) responsible for the observed activity may be desired to bring new knowledge on the cosmeceutical activity of *M. neoobscura*.

P325 Whole-cell fungal biotransformation as a tool for structural characterisation of relevant human CYP450 mediated metabolites: the case of solanidine

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The cytochrome P450 enzyme superfamily is responsible for the phase 1 metabolism of approximately 70 % of all clinically prescribed drugs [1]. Due to the highly polymorphic nature of major CYP450 isoforms – particularly CYP2D6 – there is considerable intra- and inter-individual variability in metabolic activity. Identifying specific metabolic biomarkers is therefore crucial for understanding differential drug responses and for advancing personalised medicine. In our previous studies, we showed the potential of solanidine, a steroidal alkaloid found in potatoes, and its CYP2D6-mediated metabolites as dietary biomarkers for CYP2D6 activity in a clinical setting [2]. Structural characterisation of these metabolites remains challenging due to low metabolism yields by human CYP450s. To overcome this, we employ whole-cell fungal biotransformation to produce larger quantities of its metabolites. In this study, we combine CYP-mediated biotransformation with untargeted metabolomics to investigate the phase I metabolism of solanidine. Solanidine was subjected to phase I metabolism using human liver microsomes (HLMs), and the resulting metabolite profile was analysed using high-resolution mass spectrometry, revealing 5 solanidine derived metabolites. Subsequently, whole-cell fungal biotransformation using *Botrytis cinerea* was employed as a complementary model system, revealing solanidine-derived metabolites common to both the HLM and whole-cell fungal systems [3]. This demonstrates the potential of whole-cell fungi to mimic human CYP450 metabolism and highlight the utility of whole-cell fungal biotransformations as a powerful tool for producing metabolites of interest in higher yields. This provides a scalable platform for metabolite generation and isolation, enabling comprehensive structural characterisation of metabolites produced by human CYP450 enzymes.

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P326 Miniaturized fungal cultures and metabolomics for assessing halogenated tryptophan incorporation aimed at structural diversification of bioactive scaffolds

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We recently developed a high-throughput 96-well plate platform [1] integrating parallelized miniaturized cultures and SPE to transform fungal strains into extracts compatible for antimicrobial screening and metabolomic profiling. Screening with this platform identified 102 strains active against Gram-positive and Gram-negative bacteria. Our study aimed to identify strains utilizing tryptophan in their specialized metabolites biosynthesis and assess their potential to incorporate halogenated derivatives for unnatural natural products biosynthesis. Indeed, it has been interestingly demonstrated [2] that species of the genus *Verticillium* can incorporate fluorinated tryptophan in their metabolites by precursor-directed biosynthesis. Molecular network analysis of untargeted LC-HRMS/MS metabolite profiling data of all active strains identified tryptophan-derived alkaloids in multiple extracts. Among them, we selected one strain of *Chaetomium globosum* (known to be active against *Staphylococcus aureus* [3]) and cultured it with chlorinated/brominated tryptophan. Metabolomic analysis of this strain grown with halogenated tryptophan revealed several metabolites incorporating chlorine and bromine. Based on this proof of concept, we are now identifying their structures via large-scale production for targeted isolation and full *de novo* NMR elucidation. We also are evaluating whether the 96-well platform enables rapid metabolomics-based screening of other strains' capacity to incorporate modified tryptophan (halogenated or modified differently).

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P327 A promising antiangiogenic activity of *Cynara cardunculus* L. var. *altilis* DC.

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There is considerable interest in discovering new angiomodulators from plant sources, as the inhibition of angiogenesis represents a promising strategy for

anticancer therapies. The cardoon, both wild and cultivated (*Cynara cardunculus* L., Asteraceae), is a rich source of biopharmaceutical compounds as its extracts are known to possess anti-inflammatory, antimicrobial, antioxidant, anti-thrombotic, cardioprotective, and vasodilatory effects [1]. In the present study a phytochemical investigation was performed on *C. cardunculus* L. var. *altilis* DC. (cultivated cardoon) along with the assessment of its antiangiogenic properties. The leaves of cultivated cardoon were collected and extracted (EtOH:H₂O 80:20 % v/v) (AIEt80). Quantitative analyses revealed that AIEt80 shows a remarkable content of total phenolics (200.22 mg Gallic Acid Equivalents/g dry weight) and flavonoids (62.33 mg Catechin Equivalents/g dry weight). A total of 33 compounds were identified using LC-ESI-QTOF MS/MS analysis. Regarding the main classes of compounds, flavonoids (luteolin and apigenin derivatives), hydroxycinnamic acids (mainly caffeic acid derivatives with quinic acid), and lactonic sesquiterpenes (especially cynaropicrin) were the most highly represented. Moreover, an *in vivo* assay was employed to assess the potential of AIEt80 in modulating angiogenesis, using the chick chorioallantoic membrane (CAM) as experimental model [2]. Results showed that the treatment with AIEt80 induced strong inhibitory effects on the CAM vessel growth compared to untreated control (IC₅₀ = 43.16 µg/egg). These promising insights suggest that cultivated cardoon leaves may be considered a valuable source of bioactive compounds with beneficial effects for human health and multifaceted industrial applications.

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P328 Anti-*Helicobacter pylori* compounds of *Sambucus williamsii*

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Sambucus williamsii Hance (Viburnaceae), Korean elderberry has been widely used in herbal medicine and food industry. It is known to have various pharmacological effects including antitumor, antioxidant, anti-inflammatory, and antimicrobial activities. During our search for anti-*Helicobacter pylori* compounds from natural resources, the methanol extract of the *S. williamsii* branch significantly inhibited the growth of *H. pylori*. Three phenolics and four lignans compounds were isolated from the methylene chloride fraction that had shown the most potent anti-*H. pylori* activity among the five fractions of hexane, methylene chloride, ethyl acetate, butanol, and water fractions. The chemical structures were identified as three phenolics of dihydroconiferyl alcohol (1), sylvolpinol (2) and guaiacylglycerol (3), and four lignans of boehmenan (4), lawsonicin (5) and guaiacylglycerol β-coniferyl ether (7) with a new lignan, sambucanol (6) by UV, 1H-NMR, 13C-NMR and mass spectrometry. Among the isolates, compounds 3 and 4 exhibited significant anti-*H. pylori* activity against strains 26695 and 51. Compound 3 displayed more potent anti-bacterial activity with MIC values of 3.13 and 6.25 µM, and MIC₅₀ values of 28.5 and 56.8 µM against the two strains, respectively. Their inhibitory activities were higher than those of a positive control, quercetin. Furthermore, these two compounds showed moderate urease inhibitory activity. A molecular docking study re-

vealed the high binding ability of **3** and **4** to the active site of *H. pylori* urease. These results will provide further insights into the design of more potent natural products for eradicating *H. pylori* [1, 2].

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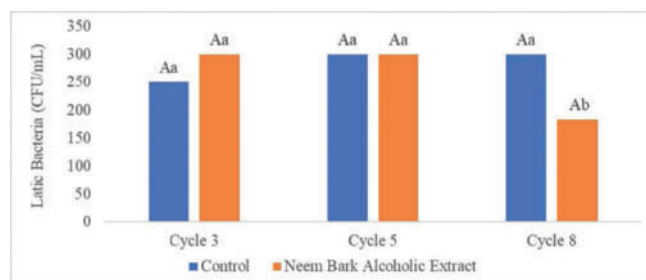
P329 Alcoholic extract of *Azadirachta indica* in the control of contaminant bacteria in ethanol fermentation

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The batch ethanol fermentation process with yeast recycling, using sugarcane as the raw material, routinely suffers from bacterial contamination in the reactors, which reduces industrial yields. In this context, neem (*Azadirachta indica* A.Juss., Meliaceae) alcoholic extract emerges as a natural alternative to commonly used synthetic antimicrobials, such as sodium monensin. This study aimed to evaluate the effect of neem bark alcoholic extract on ethanol fermentation over nine consecutive cycles. The treatments included Neem Bark Alcoholic Extract (NBAE) and a control treatment (primary), as well as the fermentation cycles (secondary). NBAE was prepared by maceration in a 1:4 ratio (bark/ethanol PA) for 48 hours. Fermentation was conducted in 500 mL Erlenmeyer flasks, utilizing 250 mL of must prepared from sugarcane juice (Brix 16%) and 7.5 g of industrial yeast CAT-11. After mixing the yeast with the substrate, the decrease in Brix was monitored until the values fell below 1%, indicating the end of fermentation, with yeast recovery achieved through centrifugation. In cycles 2, 4, and 7, NBAE was added at a dose of 50 mg/mL along with a 0.75% NaCl solution. In cycles 3, 5, and 8, the must was plated on MRS medium to quantify the lactic bacteria present. The experiment was carried out in three repetitions. It was observed that NBAE significantly reduced the number of bacteria in cycle 8, demonstrating a cumulative effect of the product in the process (► Fig. 1). It is concluded that the use of neem bark alcoholic extract controls contaminant bacteria in cyclic ethanol fermentation processes [1, 2].



► **Fig. 1** Interaction between antimicrobial agents and cycles for inoculum contamination by lactic acid bacteria. Uppercase letters indicate comparisons of treatment means within cycles, while lowercase letters indicate comparisons of cycles within each treatment.

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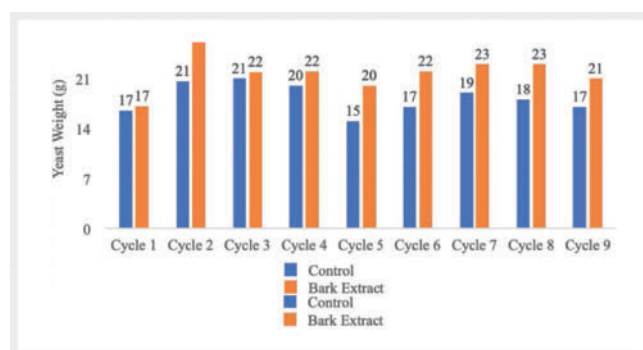
P330 Alcoholic extract of Neem (*Azadirachta indica*) promotes the yeast growth in fermentation

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In recent decades, Latin America, Africa, and Asia have emerged as major producers of bioethanol derived from sugarcane, using *Saccharomyces cerevisiae* yeast. Efforts are focused on enhancing process efficiency to increase yeast biomass production. Among these efforts, plant extracts incorporated into the process have shown potential in stimulating microbial multiplication. This study aimed to evaluate the alcoholic extract of Neem bark as a growth promoter for yeast. The treatments comprised alcoholic extract from Neem bark and a control (primary treatments), alongside nine fermentative cycles (secondary treatments). The extract was obtained from clean, dried, and ground bark through maceration in a 1:4 ratio (bark/ethanol) for 48 hours and was characterized for the presence of secondary metabolites 1. The fermentative process utilized 250 mL of wort prepared from sugarcane juice (16% Brix) mixed with 7.5 g of industrial yeast CAT-12. The Brix decrease was monitored in the fermentation process until levels dropped below 1%, marking the fermentation's end, with yeast recovery performed via centrifugation. During cycles 2, 4, and 7, the extract was added at a concentration of 30 mg/mL. The weight of the yeast was evaluated at the beginning and end of each cycle. The presence of terpenes, tannins, and phenolics in the extract was observed. The use of Neem bark extract promoted yeast growth, particularly from the fifth cycle onward, with an average increase of 20% compared to the control (► Fig. 1). It is concluded that Neem alcoholic bark extract shows promise as a yeast growth promoter in fermentative processes [1, 2].



► **Fig. 1** Interaction between antimicrobial agents and cycles for yeast weight. Uppercase letters indicate comparisons of treatment means within cycles, while lowercase letters indicate comparisons of cycles within each treatment.

Acknowledgments: the authors gratefully acknowledge financial and institutional support from the Minas Gerais Research Foundation (FAPEMIG), the Brazilian Federal Agency for Support and Evaluation of Graduate Education (CAPES), and the State University of Minas Gerais (UEMG).

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P331 Phytosterol-enriched extract from *Urtica dioica* roots as potential therapeutic agent for neuroinflammation-based diseases

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Phytosterols are natural compounds produced by plants and marine organisms, not found in humans. Structurally similar to cholesterol, phytosterols are of great importance in human diet for their cholesterol-lowering activity, together with antioxidant and anti-inflammatory properties [1]. In order to investigate possible interventions on neurodegenerative disorders, the ability of phytosterols to stimulate *de novo* neurosteroidogenesis in microglia was selected as a new approach for verifying a possible impact on cholesterol homeostasis. *Urtica dioica* L. (Urticaceae), commonly known as stinging nettle, was selected as a promising source of phytosterols. *Urtica dioica* roots has a long history for its use in traditional medicine and several studies suggested its potential in the treatment of several conditions, including prostate cancer and urological diseases [2], but its potential for the treatment of neurodegenerative disorders remained unexplored. To obtain a phytosterol-enriched phytoextract, a *n*-hexane extract was prepared by dynamic maceration of 50 g of dried roots in 500 mL at 25 °C renewing the solvent every 24 h for three times. The obtained extract (50.6 mg) was analyzed by UHPLC-ESI-MS² and the content of phytosterols was estimated to be 171.2 ± 7.4 µg/mg of dried extract by using stigmasterol calibration curve. The neurosteroidogenesis stimulation in activated human microglia mediated by phytosterols and cholesterol mechanistic pathways were investigated. The results showed that *U. dioica* root phytosterol-enriched extract efficiently stimulate the *de novo* neurosteroidogenesis influencing cholesterol clearance. These findings encourage further investigations for supporting this approach to relieve neuroinflammation in patients suffering from neurodegenerative disorders.

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P332 Chemical investigation of secondary metabolites from marine-derived fungus *Clonostachys rosea* with antiviral and antimicrobial potential

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The oceans represent a still largely unexplored environment for the discovery of new bioactive molecules. Due to their biodiversity and extreme environmental conditions, they have provided a basis for the development of a wide range of compounds with significant biological activities. Many bioactive molecules have been discovered, such as alkaloids, terpenoids and peptides, extracted from marine organisms. Marine fungi, especially in recent years, have become a significant subject of interest due to their ability to produce secondary metabolites with unique chemical structures and powerful biological properties [1]. Marine-derived strains of *Clonostachys rosea* have been found to produce peptaibols, a group of linear peptide antibiotics characterized by non-proteinogenic amino acids [2]. These compounds exhibit strong antifungal and antibacterial activity. Their unique structures and potent bioactivity make them attractive candidates for pharmaceutical development, especially in the search for new antibiotics and antifungal agents. This work is part of a multidisciplinary project, funded by Italian MIUR PRIN 2022, aimed to find new bioactive molecules from extremophilic marine fungi active against emerging or multi-resistant pathogens. This poster shows the extraction, molecular networking, bio guided assay and OSMAC approach used for the identification of a new library of peptaibols from *Clonostachys rosea* (► Fig. 1).



► Fig. 1 Workflow for the identification of new peptaibols from *Clonostachys rosea*

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P333 *Quercus cerris* L. wood chips extraction optimization: a comparative study between full factorial design (FFD) and artificial neural network (ANN)

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The search for sustainable sources of antioxidants has driven research into underutilized biomasses [1], such as *Quercus cerris* L. wood chips (QC). While established statistical methods like Full Factorial Design (FFD) are commonly

employed for process optimization, applying advanced computational techniques, mainly Artificial Neural Networks (ANN), presents a promising approach for potentially more effective extraction process modeling. This research was designed to optimize the extraction of QC antioxidant compounds, comparing the predictive effectiveness of FFD and ANN approaches. Experimental data from extractions obtained by varying temperature (20–50–80 °C), solvents (0–20–40 %EtOH/H₂O), and time (3–6–24 h) were used to build and train an ANN model. The trained ANN exhibited a higher ability to predict antioxidant activity values based on the input variables than FFD, highlighting ANN's capability to capture intricate, non-linear relationships within the results obtained from all the extraction processes. Specifically, ANN achieved a stronger correlation ($R^2 = 0.92, 0.96, 0.91$ for DPPH, FRAP, and TPC) than FFD ($R^2 = 0.83, 0.79, 0.75$ for DPPH, FRAP, and TPC) between predicted data and experimental outcomes. Furthermore, ANN demonstrated comparable ability to FFD in optimizing the extraction condition (80 °C, 24h, 40 %EtOH for FFD and 80 °C 23.47h, 39.32 %EtOH for ANN) [2]. These findings strongly support using ANN as a highly effective tool for optimizing the extraction of valuable antioxidants from plant matrices. The high degree of predictive accuracy demonstrated by the ANN model suggests its superiority in understanding and modeling the complex interactions of extraction parameters compared to traditional statistical approaches like FFD.

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P334 Phytochemical profiling of the Turkish plant *Centaurea patula*, a source of structurally diverse sesquiterpenoids

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Centaurea is a genus of flowering plants widely distributed in the Mediterranean region, belonging to Asteraceae family. Many of its species have been used for a long time in traditional medicine as herbal remedies for various purposes, it has been reported that the organic extracts of many *Centaurea* species displayed a high inhibitory activity on α -glucosidase [1], but the bioactive compounds responsible for this activity have not been identified yet. In the frame of our contribution to the characterization of biological and chemical profiles of Turkish *Centaurea* species [2], and considering the lack of detailed phytochemical studies on *Centaurea patula* DC, we carried out a comprehensive characterization of its specialized metabolites. The detailed phytochemical investigation of the aerial parts of the Turkish plant led to the isolation of 19 sesquiterpenoids belonging to the guaianolide (1–13), elemanolide (14–17), and germacrene (18–19) classes. These include the undescribed guaianolide cebelin Q (9), the previously undescribed glucosylated elemanolides 15–17, and the unprecedented pair of germacrene diastereomers patulosins A (18) and B (19). The structures of the undescribed compounds were elucidated by detailed NMR-based spectroscopic analysis, while comparison of experimental ¹³C NMR data with those obtained by DFT-based quantum chemical calculations was used to define relative configurations. Based on the activity reported for the plant extract, all the isolated compounds were evaluated for their activity on the α -glucosidase enzyme.

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P335 Isolation of polysaccharide and non-polysaccharide fractions from STW 42, *Althea officinalis* L. root extract and characterization of their anti-oxidative and wound-healing properties on human fibroblasts *in vitro*

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Whether the effect of locally acting products for treating irritative cough is a pharmacological effect or just a physical effect has been an open discussion. The root extract of marshmallow (*Althaea officinalis* L., REAo) is used in this field and in mild stomach/gut discomfort. The medicinal product STW 42 has been shown to have pharmacological effects fostering mucosal regeneration [1–4]. Mucosal wound repair depends on the recruitment of fibroblasts. The anti-oxidative, anti-inflammatory, and wound-healing properties of STW 42 and its active ingredient REAo have recently been demonstrated in MΦ and confirmed in endothelial cells [2, 3]. Anti-oxidative activity regulation may facilitate the healing process. Investigating the effect of its two main components would help to understand the action mechanism. The objective was to isolate the polysaccharide and non-polysaccharide fractions from REAo to study their anti-oxidative and pro-migratory properties on human fibroblasts. To investigate the migratory capacity by scratch assay, normal human dermal fibroblast (NHDF) were treated with STW 42, REAo, or its polysaccharide and non-polysaccharide fractions or pre-treated and co-incubated with H₂O₂ to quantify the effect on intracellular reactive oxygen species (ROS) production and mitochondrial membrane potential (MMP) stability. STW 42, REAo, or its non-polysaccharide fraction inhibited the production of H₂O₂-induced intracellular ROS and protected against MMP destabilization. STW 42, REAo, and its polysaccharide fraction improved the migratory capacity and stimulated wound closure in the scratch assay. These properties may support the benefit of STW 42 [5] in patients who are burdened with irritated laryngopharyngeal or gastrointestinal mucosa. These applications could have significant implications for the cough and gastrointestinal therapy field.

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P336 Antibiofilm effect of *Drosera rotundifolia* against uropathogenic *E. coli* strains

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Urinary tract infections (UTI), often caused by uropathogenic *E. coli*, are the most common bacterial infections worldwide [1]. The increasing prevalence of multidrug-resistant pathogens and their ability to form biofilms make effective treatment difficult and contribute to recurring infections [2]. Although herbal medicines such as bearberry leaves are already used in UTI therapy [3], there is still a need for innovative treatment. *Drosera rotundifolia*, a carnivorous plant and traditionally used for respiratory ailments, contains antibacterial naphthoquinones and flavonoids with spasmolytic properties [4, 5]. In this study, crude extracts, fractions, and isolated compounds were tested in a bioactivity-guided approach for their efficacy against uropathogenic *E. coli* strains. To gain deeper insights into the underlying mechanisms of the observed anti-biofilm activity, proteomic analyses were carried out. The data showed that treatment with *Drosera rotundifolia* led to an increased abundance of proteins involved in iron uptake, suggesting that the plant extract may induce iron stress in bacteria. Iron is an essential element for both bacterial metabolism and virulence. Interfering with iron-dependent mechanisms may therefore represent a promising approach to weaken bacterial persistence and pathogenicity. This effect may be attributed to the ability of the plant's flavonoids to chelate iron. The iron-binding capacity of *Drosera rotundifolia* flavonoids was confirmed using a Chrome Azurol S assay. Consequently, the inhibition of iron metabolism by flavonoids may play a crucial role in reducing biofilm formation. These findings highlight the potential of *Drosera rotundifolia* as a source of antivirulence agents and offers an innovative approach for the treatment of uncomplicated, recurrent urinary tract infections.

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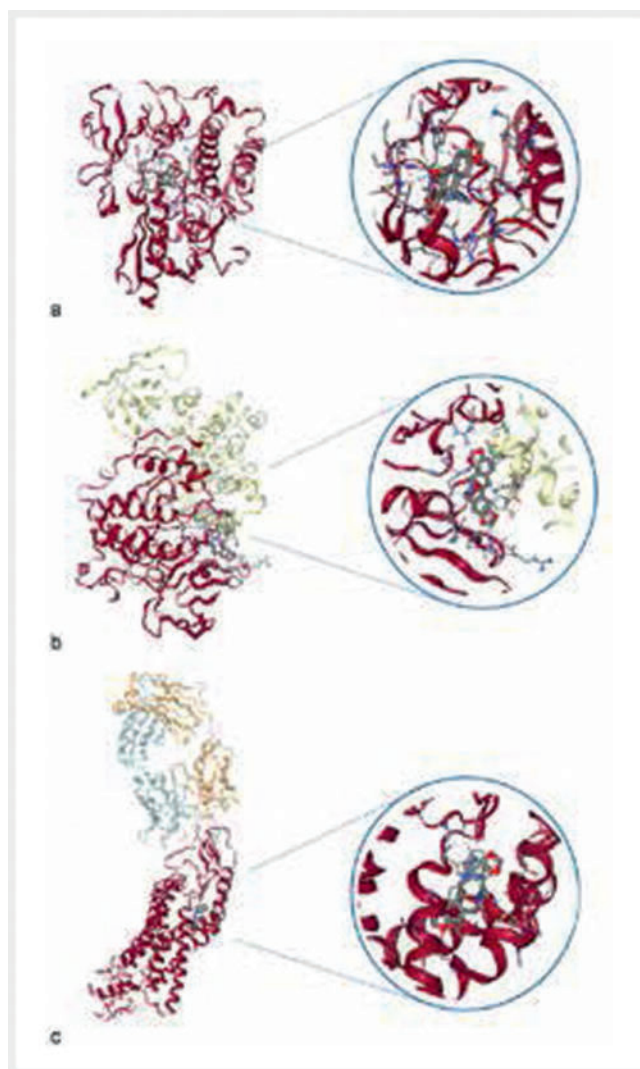
P337 Protopine as a multitarget modulator of glucose homeostasis: An *in silico* approach

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Protopine is a benzyloquinoline alkaloid found in several Papaveraceae species with known anti-inflammatory and neuroprotective effects [1]. However, its potential as an antidiabetic agent remains underexplored. This study aimed to evaluate the interaction profile of protopine with multiple key proteins involved in glucose homeostasis using *in silico* molecular docking. Docking simulations were conducted on eight diabetes-related protein targets: PPAR γ (2PRG), AMPK (5ISO), α -glucosidase (3TOP), PTP1B (1T49), insulin receptor kinase (1IRK), GSK-3 β .1), suggesting a potential role in modulating both insulin signaling and incretin pathways, which are clinically targeted by GLP-1 receptor agonists in diabetes treatment (► Fig. 1). Strong interactions were also observed with PPAR γ , α -glucosidase, and AMPK, supporting a multifaceted mechanism that may involve enhanced insulin sensitivity, inhibition of glucose absorption, and improved energy metabolism [3]. These preliminary findings highlight protopine as a possible lead compound for further pharmacological investigation in the context of type 2 diabetes, although these values should be interpreted with caution due to limitations of *in silico* scoring functions. Further *in vitro* and *in vivo* studies are needed to validate its therapeutic potential [2].



► **Fig. 1** Molecular docking visualization of protopine bound to key antidiabetic target proteins. (a) Binding pose of protopine in the active site of AKT1 (PDB ID: 3O96). (b) Binding interaction of protopine with GSK-3 β (PDB ID: 1Q5K). (c) Binding conformation of protopine at the GLP-1 receptor site (PDB ID: 6LN2)

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P338 Effects of genetic and environmental variation on strawberry anthocyanins

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Anthocyanins are naturally occurring phenolic compounds from the flavonoid class, which are commonly found in berries. These compounds are known to provide many human health benefits due to their antioxidant and anti-inflammatory properties. In this study, we investigated how genotypic and environmental variation influences anthocyanins in strawberry fruits. We used high-performance liquid chromatography to quantify several dominant anthocyanin compounds in fruits of six strawberry (*Fragaria x ananassa*) cultivars and wild strawberries (*Fragaria virginiana*) grown in a raised bed common garden experiment in Michigan, USA. Additionally, we examined how genotypic and environmental variation affected anthocyanins of strawberries grown on ten farms across a rural-urban gradient in the Detroit metropolitan area. We found that total anthocyanin concentrations were comparable in cultivated and wild strawberry fruits, with notable variability in both total and individual anthocyanins across cultivars and sites. Anthocyanin content was positively associated with mean nighttime. Temperature and cultivar were key predictors of total anthocyanin content, explaining about 20% of variation. These results highlight the importance of growing conditions and suggest anthocyanin production may be enhanced by slightly warmer nighttime temperatures in our northern climate, especially at sites which also had comparatively lower daytime temperatures. This study adds to our growing understanding of phenotypic plasticity in strawberry phytochemistry and functional traits [1–3]. Future research will evaluate the significance of observed variation in strawberry phenolic and terpenoid profiles on antioxidant capacity and antimicrobial properties.

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P339 Exploration of antiparasitic activity of *Miconia theaezans* leaf extracts and isolated compounds against *Plasmodium falciparum*

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Malaria remains one of the most devastating infectious diseases globally, causing hundreds of thousands of deaths annually, particularly in tropical and sub-tropical regions. Despite advances in prevention and treatment, the growing

resistance to conventional antimalarial drugs emphasizes the urgent need for novel therapeutic agents [1, 2]. This study investigates the antiparasitic potential of *Miconia theaezans* (Melastomataceae) leaf extracts and isolated compounds and fractions. The antiparasitic activity of various extracts, chromatographic fractions, and isolated compounds was evaluated in vitro against *Plasmodium falciparum* FCR-3, showing significant inhibition of parasite growth with IC₅₀ values below 100 µg/mL. Bioassay-guided fractionation led to the isolation of two compounds, whose structures were elucidated by NMR and MS analyses. One of the isolated compounds demonstrated notable antiparasitic activity. Cytotoxicity was assessed in 3T3 fibroblasts, confirming minimal toxicity to mammalian cells. These findings support the antiparasitic potential of *M. theaezans*, both in crude extracts and in specific isolated compounds [3]. The ongoing identification and profiling of secondary metabolites through LC-MS aim to further clarify the chemical basis of the observed bioactivity. This work contributes to the discovery of novel lead compounds for the development of future antimalarial therapies.

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P340 Sustainable extraction of apple pomace to recovery the bioactive compounds with pro-health properties

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About 44% of the total amount of waste generated in the world is food and green waste [1] including by-products from the agri-food industry such as the apple juice. The disposal of the apple pomace can cause serious environmental problems or even public health hazards if it is not managed or processed properly. Nowadays, the recovery rate of AP is quite low and insufficient. Therefore in this work we examined different ways of apple pomace extraction including conventional and sustainable methods of extraction i.e. ultrasound-assisted (UAE), enzyme-assisted (EAE), β -cyclodextrin-assisted (CAE) and pressured solvent extraction (PSE). The extracts were examined by the chemical composition (polyphenols, terpenoids, fatty acids, vitamins, minerals), antioxidant properties (in DPPH, ABTS, FRAP, CUPRAC, lipid peroxidation spectrophotometric assays). The biocompatibility of the extract and the cellular antioxidant potential of extracts were examined on human epidermal cells (HaCaT). The obtained results showed that the way of extraction extremely affects the chemical composition and biological activity of apple pomace extracts. The obtained extracts are valuable biomaterials that can be applied in agri-food-health industry [2].

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P341 Biological activity studies on *Hyssopi Herba* from Turkey

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Hyssopus officinalis L. (Hyssop) is one of the most popular herbs, mainly distributed in the Eastern Mediterranean to Central Asia. *Hyssopi herba* is used in tea blends for cough relief, antispasmodic effects, and to relieve catarrh [1]. The plant has recently attracted attention due to its phenolic content and promising biological activities, such as antimicrobial and antioxidant effects [2]. In the present study, the total phenolic and total flavonoid content were determined in two different commercial samples of *Hyssopi herba*. Additionally, neuroprotective activity and anticholinesterase activity tests were conducted as part of our study. Cell viability was evaluated using an MTS/PMS assay in a cellular model of oxidative stress after exposure to extracts at various concentrations (1–100 µg/mL), and the anticholinesterase activity studies were carried out using Ellman's method. Methanolic extracts were prepared, and yields of extracts (H1 and H2) were calculated as 11.94 % and 24.06 %, respectively. In extract H1, total phenols and total flavonoids were determined to be 80.188 ± 0.495 mg GAE/g and 71.929 ± 2.538 mg QE/g, whereas in extract H2, total phenolic and flavonoid contents were found to be 282.394 ± 14.036 mg GAE/g and 49.814 ± 2.420 mg QE/g, respectively. The methanolic extracts (H1 and H2) did not show noticeable anticholinesterase inhibitory activity (AChE 37.7 and 11 %; BChE 16 and 23 %, respectively). Neuroprotection by the extracts in the presence of hydrogen peroxide was indicated by a significant reduction of cellular loss at concentrations less than 10 µg/mL ($p < 0.05$).

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P342 HPLC studies on rosmarinic acid and chlorogenic acid in some Lamiaceae tea plants

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Recently, the unstoppable side effects of synthetic drugs have further emphasized medicinal plants as a reliable source of treatment. The Lamiaceae family, one of the most widespread plant families, contains a wide variety of plants that are very active in terms of biological and medicinal applications. This study aimed to determine the amounts of rosmarinic acid and chlorogenic acid, which are valuable phenolic acids, in extracts obtained by aqueous infusion which is used as the herbal tea and extraction with 80 % EtOH from 12 Lamiaceae species growing naturally or cultivated in Turkey by High-Performance Liquid Chromatography (HPLC). 12 Lamiaceae species were collected from the Turkish flora [*Salvia rosmarinus* (*Rosmarinus officinalis* L.), *Melissa officinalis* L., *Origanum onites* L., *Tymus vulgaris* L., *Mentha spicata* L., *Mentha x piperita* L., *Lavandula x intermedia* Emeric ex Loisel., *Salvia officinalis* L., *S. sclarea* L., *S. triloba* L., *L. stoechas* L. subsp. *stoechas*, *Ocimum basilicum* L.]. The chromatographic method used was properly validated according to the procedures specified in ICH guideline Q2(R1) on validation of analytical methods (3). As part of the method validation, validation parameters such as sensitivity, linearity, accuracy, robustness, precision, specificity, and system suitability tests were examined and it was determined that the values obtained were within acceptable limits according

to the results of the same-day and different days. According to the results, it was found that the plant extract with the highest rosmarinic acid content was *Melissa officinalis* L. 80 % EtOH extract (3.0 mg/g of dried plant) and the plant extract with the highest chlorogenic acid content was *Lavandula x intermedia* 80 % EtOH extract (0.2 mg/g of dried plant). Phytochemical investigation and screening on biological activity of selected Lamiaceae species are ongoing. These plants are valuable sources of rosmarinic acid and will be promising reservoirs for the future production of pure rosmarinic acid [1–3].

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P343 The potential antioxidant properties of Rosemary extracts

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Research into new bioactive compounds is attracting the interest of consumers and the global market for their positive impact on personal health and well-being. As a result, the demand for ecofriendly and safe food products is growing [1]. In particular, Aromatic and Medicinal Plants (AMPs) from Mediterranean basin are important source of phytochemicals and essential oils (EOs). Among the AMPs, *Rosmarinus officinalis* L., commonly known as rosemary, belongs to the Lamiaceae family. Rosemary can be cultivated or grow wild as an evergreen shrub in the Mediterranean region and the leaves are usually used fresh or dried to flavor foods and beverages [2]. Nowadays, the European Commission authorized the use of rosemary extracts and EO in food preservation, cosmetics, pharmaceuticals, natural therapies, and alternative medicine [1, 2]. The aim of this study was the characterisation of the polyphenolic and terpenic fractions of a wild *R. officinalis* variety harvested in the province of Naples (Italy). The polyphenols were extracted from leaves by means of 80 % ethanol, at 70 °C for 60 minutes and the EO extraction was performed using hydro-distillation. Furthermore, the polyphenolic extracion was also carried out on the rosemary post-distillation solid residue, a biomass with no apparent commercial value. As a result, the phytochemical potential of EO and rosemary extracts is exploited here and several compounds with valuable antioxidant function are present. Furthermore, these results fall within the circular economy strategy for sustainable valorisation of agricultural waste as a source of useful bioactive compounds.

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P344 *Calendula* spp. flowers as pleasant reservoir of valuable phytochemicals

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The genus *Calendula*, belonging to the Asteraceae family with about 20 species native of the Mediterranean region, has been used from Greeks, Indian and Arabic cultures since ancient time [1]. Nowadays, calendula is worldwide cultivated as ornamental, therapeutic and health-promoting plant. Particularly, flowers are commonly utilized for medicinal, pharmacological and wellness purposes, owning a wide spectrum of biological activities such as antioxidant, antibacterial, antifungal, antiviral. All these properties are related to the content of several classes of secondary metabolites such as polyphenols, flavonoids, tannins, terpenes, sterols, saponins, carotenoids [1]. In Italy, *Calendula officinalis* is the most cultivated and *Calendula maritima* is endangered, growing in some coastal areas of Sicily. This study is carried out on their flower heads, lyophilized and extracted with a green solvent. In particular, a 60 % ethanol extraction was carried out with ultrasonic support, followed by partitioning of the extracts with increasing solvent polarity (petroleum ether, dichloromethane and butanol). The butanolic extracts were then subjected to chromatographic separation on a Sephadex LH-20 column [2]. The bioassays conducted on all fractions allowed to identify saponins and phenolic compounds as leading classes of bioactive compounds, both in *C. officinalis* and in *C. maritima* flowers. This research is propaedeutic for the overall chemical and biological characterisation of the health-promoting molecules of *C. officinalis* and *C. maritima* flowers. In addition, these data will be useful for promoting the protection and valorization of Mediterranean plant species with many potential applications in different fields for human well-being.

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P345 Discovery of novel cytochalasin derivatives from an endophytic fungus isolated from *Senecio doronicum*

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Plant fungal pathogens such as *Botrytis cinerea* pose a significant threat to global agriculture and forestry, causing crop yield losses, quality degradation, and even jeopardizing food security. It is estimated that fungal diseases account for annual crop losses of approximately 10 % to 28 % [1, 2]. Moreover, with the exacerbation of climate change, the spread and severity of fungal diseases are further increasing [3, 4]. Therefore, the search for novel antifungal agents to combat these phytopathogens is of great importance. In a screening campaign for the discovery of antifungal substances from endophytic microorganisms of alpine plants against *B. cinerea*, we have identified an endophytic fungus (*Ascomycota* phylum) from the leaves of *Senecio doronicum*, displaying a promising bioactivity (MIC = 25 µg/mL). The fungus was cultured on rice, followed by

extraction and bioactivity-guided fractionation. Feature-Based Molecular Networking (FBMN) analysis of the LC-MS/MS data identified a cluster that correlated with antifungal activity. This led to the isolation of seven cytochalasin derivatives, four known derivatives, cytochalasin A (1), B (2), F (3), and deoxaphomin (4) and three new analogues of cytochalasin A, cytochalasin A1 (5), A2 (6); and A3 (7). The structural characterization was performed using 1 & 2D NMR and HRMS, and their absolute configuration was established using electronic circular dichroism. Antifungal evaluation of all the compounds isolated showed that cytochalasins A and F exhibited strong activity against *B. cinerea* (MICs = 3.125 µg/mL and 6.25 µg/mL, respectively). The results obtained illustrate the potential of alpine plant endophytes as promising sources of bioactive compounds.

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P346 Antifungal secondary metabolites of an endophytic fungus of *Picea abies*

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Botrytis cinerea is one of the most common agricultural pathogens which imposes significant economic loss of between 10 to 100 billion dollars annually [1]. Recent studies indicate that *B. cinerea* can develop resistance against conventionally used fungicides. Therefore, there is an increased demand for discovery of new potential fungicides to combat *B. cinerea*. An alternative approach for discovery of new antifungal agents against this emerging pathogen is utilizing the natural products derived from fungal endophytes. These symbiotic microbes are underexplored sources of bioactive substances and therefore hold a great potential for discovery of novel antifungals [2]. In this study, we aimed for isolation and identification of the bioactive metabolites of *Paraleptosphaeria* sp., an endophytic fungus isolated from the needles of *Picea abies*, and assessment of their antifungal activities against *B. cinerea*. The fungus was up-scaled on a rice medium, then extracted using ethyl acetate. Subsequently, the extract was systematically separated by various chromatography techniques using silicagel, Sephadex LH-20, and C18, which ultimately yielded seven compounds. The structures of the compounds isolated were identified by 1&2-D NMR spectroscopy methods and LC-ESI-HRMS, resulting in three new (1-3) and three known (4-6) chromophilones, and a new versiol-like terpenoid (7). The absolute configuration of the compounds was established by comparison of experimental and calculated circular dichroism spectra. Ultimately, pure compounds were evaluated for their bioactivities against *B. cinerea*, among which compound 7 displayed promising antifungal activity. According to the results obtained, *Paraleptosphaeria* sp. can be considered as a promising source of unexplored secondary metabolites with potential antifungal activities.

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P347 Chemical and biological investigation of rhizospheric *Streptomyces* from *Ebenus sibthorpii*

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The rise of multi-drug resistant pathogens (MDR) underscores the urgent need for developing new antibiotics. Natural products, particularly bacteria-derived metabolites, are a promising source of bioactive compounds with significant antimicrobial potential. Plant-associated microbiomes, especially in the rhizosphere, are integral to plant protection and growth, primarily through antibiotic production. Among these, *Streptomyces* species, abundant in rhizospheres, are renowned for their robust antimicrobial and anti-fungal compound synthesis [1]. This research aimed to isolate and evaluate *Streptomyces* strains from the rhizosphere of the *Ebenus sibthorpii* plant as potential sources of antimicrobial metabolites. *Streptomyces scopiformis* was identified through molecular techniques, selected based on significant antibiotic and antifungal activity against indicator organisms. Laboratory and pilot-scale cultures of this strain were undertaken, and extensive chemical analysis of both culture micelles and nutrient materials was performed using LC-HRMS/MS, identifying a total of 100 compounds. The centrifugal partition extraction technique was crucially employed to efficiently separate and fractionate nutrient substances, demonstrating excellent separation capability and significant time efficiency. Additionally, acid precipitation and sedimentation methods with water and acetonitrile facilitated further fractionation. Preparative thin-layer chromatography was subsequently utilized for additional purification and identification. Bioactivity assays against *Candida albicans* and *Staphylococcus aureus* indicated that specific nutrient fractions exhibited notable antimicrobial activities. Overall, this study effectively validated the methodology and experimental protocols developed, establishing a robust framework for exploring and characterizing the antimicrobial chemical content of *Streptomyces*-derived metabolites from rhizosphere microbiomes.

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P348 α -Glucosidase inhibitory and antioxidant activity of selected *Polygonum* species

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Polygonum is a diverse genus within the Polygonaceae family, comprising about 300 species distributed in temperate, tropical and subtropical regions. *Polygonum* species are traditionally used to treat a variety of conditions such as inflammation, cardiovascular disease, infections and neurodegenerative disorders due to their rich phytochemical profile, which includes terpenes, flavonoids, phenolic acids and stilbenes. Despite their widespread distribution and use, many *Polygonum* species are poorly studied. While some of them have well-documented pharmacological effects, others are chemically unexplored or have not been thoroughly studied biologically [1, 2]. Therefore, our research aimed to investigate the polyphenolic composition and biological properties

of *Polygonum* species growing in Croatia. The total content of phenolic acids (0.42–6.03%), flavonoids (0.01%–0.90%) and tannins (0.14%–3.13%) in dried aerial parts and roots was determined spectrophotometrically. Quercetin glycosides, caffeic acid derivatives and stilbenes were determined in ethanolic extracts of *Polygonum* species by RP-HPLC DAD. The extracts showed the ability to inhibit α -glucosidase activity (IC_{50} = 33.92–1645.67 μ g/mL). In addition, the significant DPPH (IC_{50} = 65.63–310.36 μ g/mL) and NO radical scavenging activity (IC_{50} = 13.06–171.86 μ g/mL) and reducing power (IC_{50} = 15.69–51.72 μ g/mL) of the tested extracts were determined. Our results suggest that the selected *Polygonum* species possess promising hypoglycemic and oxidative stress reducing properties due to their potent α -glucosidase inhibitory activity and antioxidant properties.

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P349 Exploration of South African biodiversity associated with indigenous knowledge for the discovery of natural ingredients as potential drug leads

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South Africa, recognized as one of the world's biodiversity hotspots, with a rich tapestry of indigenous knowledge systems, presents unique opportunities for sustainable bioprospecting and development of innovative natural ingredients. The research highlights a multidisciplinary approach using modern scientific methodologies to harness South Africa's rich biodiversity and the wealth of associated indigenous knowledge for the discovery, development, and commercialisation of new natural products. Surveys were conducted at communities in South Africa and the traditional uses of identified medicinal plants were recorded. Plants were collected, dried, ground and extracted using aqueous and organic solvents. Each extract was fractionated using solid-phase extraction to produce seven corresponding fractions in 96-well plate format for high throughput screening. Extracts and fractions were tested *in vitro* for cytotoxicity and efficacy to identify ingredients with medicinal properties, including analgesic, anticancer, antidiabetic, antiviral and antimicrobial properties. The fractions showing good biological activity were analysed by ultra performance liquid chromatography – quadrupole time-of-flight – mass spectrometry to tentatively identify the compounds responsible for bioactivity. Antidiabetic ingredients, from *Buddleja saligna* Willd. (Scrophulariaceae) and *Crassula muscosa* L. (Crassulaceae), have been identified through this approach. Seventy two samples were screened for enzyme inhibition activity against α -amylase and α -glucosidase, and glucose-uptake activity in liver cells. Two extracts and their corresponding fractions showed good antidiabetic activity (IC_{50} < 10 μ g/mL). Research is ongoing to isolate and identify the compounds responsible for the bioactivity through bioassay-guided fractionation using semi-preparative high performance liquid chromatography. This will be followed by the development of standardised ingredients and products.

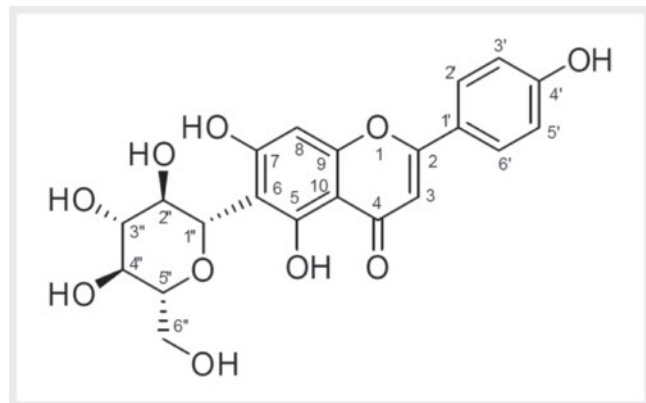
P350 *Passiflora edulis* Sims as a source of bioactive metabolites: optimization of isolation procedures

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Nowadays different food and pharmaceutical formulation of *Passiflora edulis* Sims are available on the market. This tropical climbing plant, also known as “passion fruit”, has been used since ancient times to produce traditional remedies in South America [1]. The aim of this study has been to produce and characterize hydroalcoholic extracts of leaves of *P. edulis* Sims using different solid-liquid extraction techniques: a) Maceration or infusion; b) Ultrasound Assisted Extraction (UAE); c) Rapid Solid-Liquid Dynamic Extraction (RSLDE), an innovative technique based on the “Naviglio principle” [2]. The extraction kinetic of the plant matrix in different hydroalcoholic solutions for each techniques listed above has been evaluated through the optimization of a kinetic exponential function to realize a comparison between them. Moreover, the extracts have been analysed using the Folin-Ciocalteu and DPPH (2,2-diphenyl-1-picrylhydrazyl) assays. Under the optimal conditions identified for each technique, the comparison highlighted a drastic reduction in extraction time made possible by technological advancement, without altering the yield. Subsequently, the extracts were purified using different chromatographic techniques to isolate the main secondary metabolite of *P. edulis* Sims. This compound has been identified as isovitexin (► Fig. 1) [3] using mass spectrometry, optical methods and various NMR techniques and its amount quantified by HPLC-UV in the various extracts. The demonstrated high levels of isovitexin in all extracts emphasize the possibility of employing ecologically sustainable extractive methods to obtain this *fine chemical*.



► **Fig. 1** Chemical structure of isovitexin, a powerful antioxidant isolated from *Passiflora edulis*.

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P352 Deciphering the antidiabetic potential of *Eugenia uniflora* L. leaf via α -amylase and α -glucosidase inhibition

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Eugenia uniflora L. leaf (EUL) is implicated in the African traditional medicine as a remedy for diabetes, obesity and other metabolic diseases without much scientific knowledge of its therapeutic action and active ingredients [1]. This study evaluated the antidiabetic potential of EUL by isolating its putative α -amylase and α -glucosidase inhibitors. The EtOH-H₂O (80:20) extract was solvent-partitioned into n-hexane, DCM, EtOAc, and n-BuOH fractions. A bioassay-guided chromatographic fractionation of the most active EtOAc fraction led to isolation of two compounds. The structure elucidation of the compounds were assisted by 1D- and 2D NMR spectroscopy as well as high-resolution mass spectrometry, affording flavone glycosides, namely: Myricetin-3-O-rhamnoside (1); and its isomer described as Unifloratrin (2). The extract, EtOAc fraction, 1 and 2 exhibited IC₅₀ values of 67.1 $\pm$ 2.5, 49.4 $\pm$ 2.9, 25.6 $\pm$ 1.8 and 33.0 $\pm$ 2.2 μ M against α -amylase enzyme, suggesting that fractionation has enhanced the bioactivity of the plant. Similarly, 1 demonstrated notable inhibition against α -glucosidase (IC₅₀ = 19.3 $\pm$ 2.6 μ M), which was less effective to the Metformin standard drug (11.5 $\pm$ 1.5 μ M) but comparable to Acarbose (17.1 $\pm$ 1.3 μ M). It can be concluded that EUL actually contains α -amylase and α -glucosidase inhibitors that may help to modulate postprandial blood glucose elevations in diabetic conditions. The study findings also validate the ethnomedicinal claim of EUL as a remedy for diabetes and other metabolic ailments.

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P353 Exploring the neuroprotective potential of *Cecropia peltata*: Insights from in vitro and *Drosophila melanogaster* models

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Neurodegenerative disorders represent a major global health burden due to their increasing incidence and the current lack of effective disease-modifying therapies [1, 2]. The multifactorial etiology of these disorders underscores the therapeutic potential of natural products, particularly those exhibiting multi-target activity through antioxidant and anti-inflammatory mechanisms [3, 4]. In this context, we investigated the neuroprotective effects of *Cecropia peltata*, a medicinal plant traditionally used in Colombia for the management of tremors and symptoms linked to neurodegenerative disorders, such as Parkinson's disease. Ethanolic extracts and derived fractions of *C. peltata* were obtained and evaluated for their antioxidant activity using DPPH, Folin-Ciocalteu, and FRAP assays. The total extract and methanolic fraction displayed potent antioxidant capacity, with IC₅₀ values below 50 ppm. The neuroprotective effects of the most promising antioxidant samples were subsequently assessed in acrylamide- and paraquat-induced neurotoxicity models in SH-SY5Y cells and *Drosophila melanogaster*. Both models revealed that *C. peltata* extract and fraction significantly attenuated oxidative stress-induced cellular damage. In *Drosophila*, post-exposure treatment with an acute dose of 1000 ppm (three days) improved the locomotor function in acrylamide-exposed flies, probably through the upregulation of endogenous antioxidant systems. These findings

highlight the neuroprotective potential of polar metabolites from *C. peltata*, supporting their ethnopharmacological use and promoting further investigation into their chemical composition and mechanistic pathways of action.

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P354 *Perovskia atriplicifolia* Benth (Brazambal Balochil), a source of antioxidant abietane diterpenes

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Perovskia atriplicifolia Benth is a perennial and aromatic plant widely distributed in the Sistan and Baluchestan region of Iran. In Persian, *P. atriplicifolia* is known as “Brazambal balochil”, and is traditionally used as a cooling medicine in the treatment of fevers, leishmaniasis, headache, rheumatic pains, and toothache in Iranian folk medicine [1].

Previous investigations on *P. atriplicifolia* were focused on the chemical composition of its essential oil, characterized by GC-MS [1]. To the best of our knowledge, little is known about the chemical composition of the non-volatile extracts of *P. atriplicifolia*. Herein, an analytical approach based on LC-ESI-Q-Exactive-MS/MS, in negative ion mode, was carried out to obtain a chemical profile of the EtOH extract of *P. atriplicifolia* aerial parts. The LC-MS analysis of the EtOH extract showed the presence of 19 compounds, tentatively identified, based on their accurate masses, characteristic fragmentation pattern, and literature data. To unambiguously assign compounds corresponding to these peaks and to discriminate among structural isomers, isolation followed by structure elucidation by 1D and 2D NMR experiments of isolated compounds was carried out. Compounds belonging to the classes of phenolic acid derivatives, diterpenes and triterpenes have been characterized. To our knowledge, this is the first report of caffeic acid ethyl ester, and urs-11-en-28,13 β -olide in the genus *Perovskia*.

Furthermore the obtained results highlighted how the EtOH extract of *P. atriplicifolia* aerial parts represents a good source of the well-known antioxidant abietane diterpenes carnosic acid and carnosol [2] along with related metabolites namely epirosmannol, epi-isorosmannol, 1 α -hydroxydemethylsalvicanol, 7-O-methoxyrosmannol, royleanonic acid and 12-methoxycarnosic acid. Considering the well-known antioxidant properties of carnosol and carnosic acid, the antioxidant activity of the EtOH extract of the aerial parts of *P. atriplicifolia* and of the main abietane diterpenes were tested in Caco-2 cell line. Cells were treated with the prooxidant agent tert-butyl hydroperoxide (TBH) and ROS production was measured through the use of a fluorescent probe. Pretreatment with the EtOH extract, at non toxic concentrations (0.5–25 μ g/ml), counteracted TBH-induced alteration of cellular redox status, with a significant and dose-dependent decrease of ROS production. All of the abietane diterpenes tested (0.5–25 μ M) showed the ability to prevent ROS formation, being

7-O-methoxyrosmannol the most effective, even more than carnosol and carnosic acid.

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P355 New insights into hemp essential oils: Chemical, antimicrobial, and sensory profiling of underexplored genotypes

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Cannabis sativa L. is a versatile crop with growing relevance across multiple sectors. Its genetic and phenotypic variability, although challenging, provides valuable opportunities to develop improved cultivars tailored to specific purposes. Hemp inflorescences, the major by-product of hemp processing, are rich sources of bioactive compounds, including essential oils (EOs). EOs are complex mixtures of volatile compounds, primarily composed of terpenes and terpenoids, which in addition to being responsible for the aroma, also contribute to the biological activity [1–3].

The present study aimed to chemically characterize the EOs obtained from four underexplored industrial hemp genotypes (E, H, M, and N) selected by the CREA-CI (Bologna), and to assess their antimicrobial and sensory properties. All EOs showed high percentages of mono- and sesquiterpene hydrocarbons and oxygenated sesquiterpenes, with major differences in the relative abundances of key compounds. Antimicrobial activity, evaluated by MIC and MBC assays against six ATCC strains, evidenced a stronger antibacterial effects of all EOs against Gram-positive bacteria, and the best outcomes were obtained by the genotype N against *Listeria monocytogenes*. Sensory evaluation revealed good scores of olfactory pleasantness for all samples, with notable differences in perceived notes.

Overall, the EO of genotype N stood out for the greatest antimicrobial activity, high hydrodistillation yield, and pleasant fresh-vegetable and fruity aroma. Likewise, the EO of genotype M showed good antimicrobial activity and the most appreciated floral and balsamic notes.

Considering their promising features, the EOs of N and M could be selected for further studies to evaluate their activity against *L. monocytogenes* field strains for possible applications in the food industry.

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P356 Evaluation of antioxidant compounds in fruits of Long Shelf Life (LSL) tomato landraces during post-harvest

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The tomato (*Solanum lycopersicum*) plays a very interesting role in human health thanks to its content of functional compounds. In particular, some of these compounds, such as flavonoids, carotenoids, phenolics and vitamin C, are very important for their health properties [1]. In the Mediterranean region, several tomato landraces, called “da serbo”, are characterised by fruits with a long shelf-life and high levels of sugars and antioxidant compounds [2].

In this study, the antioxidant capacity and the antioxidant profile of the fruit pericarps were compared in two LSL tomato landraces (“Lucariello” and “Corbarino”) (► Fig. 1) cultivated in the Campania region (Southern Italy), which are characterised by a different prolonged shelf-life after harvest [3]. In order to gain new insights into the molecular mechanisms controlling the production of antioxidant compounds during post-harvest in LSL tomato landraces, the expression profiles of key genes involved in their biosynthetic pathways were also studied.



► **Fig. 1** Fruits of LSL tomato landraces, “Corbarino” (a) and “Lucariello” (b), at harvest.

High-resolution LC-MS phytochemical analysis revealed that the polyphenolic profile remains stable after harvest, with no significant decline in key compounds such as dihydrokaempferol, rutin, taxifolin and naringenin. Similarly, beneficial carotenoids, including luteolin, zeaxanthin and beta-carotene are effectively preserved over time. These results highlight the resilience of these metabolites during storage and underline their potential to maintain nutritional value and health benefits post-harvest. Furthermore, our results showed that the expression profiles of key genes involved in flavonoid and carotenoid pathways during post-harvest were genotype-specific, strongly influencing the nutritional quality and health benefits of ‘da serbo’ tomato landraces.

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P357 Unveiling the hidden potential of hop extraction side streams: Chemical profiling and bioactivity

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The processing of hops (*Humulus lupulus* L.) for the brewing industry generates side-products that vary depending on the applied extraction method [1]. These by-products are currently used – if at all – primarily as feed additives. However, the by-products still contain significant amounts of organic compounds. Due to the extensive industrial processing of hops, these by-products may contain rare or previously unidentified compounds in significant concentrations, which would likely go unnoticed or remain unrecovered by conventional whole-plant laboratory extraction techniques.

In continuation of our studies on hop compounds [2], the objective of the study was to characterize a hop-derived side stream to evaluate the potential as a high-value by-product.

The extracts were analyzed using LC-PDA-HRESIMS, GC-MS, and NMR. In addition, cellular protection assays were performed using HT1080 cell line to explore application potential in skin care and SH-SY5Y cells to assess potential neuroprotective effects.

The fractions contained a substantial amount of multifidols, humulones, lupulones, flavonoids, and prenylated flavonoids, dependent on fractionation. This hop by-product thus represents a promising and previously underutilized source of rare bioactive compounds, offering new perspectives and value-added applications in the pharmaceutical or nutraceutical industry.

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P358 High-performance thin layer chromatography profiling and antimicrobial activity of *Lobostemon fruticosus* (L.) H.Buek

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Lobostemon fruticosus (L.) H.Buek is a woody, evergreen shrub native to South Africa’s Cape region. Traditionally, the aerial parts of this plant have been used by indigenous communities to treat various skin ailments. While previous studies have documented the chromatographic profiles of the plant’s secondary metabolites and identified key marker compounds for quality control, there has been no prior investigation into the chemotypic variation across different populations of this species. This study aimed to compare the chemical profiles

of aqueous methanolic extracts ($n = 24$) collected from eight distinct South African locations using high-performance thin layer chromatography (HPTLC), and to assess their antimicrobial activity against seven skin-related pathogens. The extracts (80 % aqueous methanol) prepared from the aerial parts were profiled using a semi-automated HPTLC system. Distinct bands were isolated, dissolved in methanol, and analysed using ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) for compound identification. Antimicrobial activity was evaluated using the broth microdilution method against *Staphylococcus aureus*, *S. epidermidis*, *S. haemolyticus*, *Enterobacter hormaechei*, *Streptococcus pyogenes*, and *Pseudomonas aeruginosa*. The HPTLC profiles were largely consistent across populations, with globoidnan A and B, rabdosiin, rosmarinic acid, and rutin identified as major compounds, showing quantitative variation among the samples. This variation was confirmed by UPLC-MS analysis in tandem with chemometrics, where subtle chemotypic variation was observed ($R^2X_{cum} = 0.496$). Moderate antimicrobial activity was observed against all tested pathogens, with minimum inhibitory concentrations (MICs) ranging from 2 to 4 mg/mL. These results highlight the need for further research on this medicinal plant.

P359 Synthesis and evaluation of the cytotoxic activity of molecular hybrids and Resomer® RG 504 H nanoparticles loaded with triterpenes isolated from Mexican plants

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The synthesis of hybrid compounds has been shown to increase tumor-targeted cytotoxic activity, improving the potency of therapy [1]. Furthermore, hybrid compounds overcome many disadvantages of single-dose drugs, such as cellular resistance to monotherapy, adverse effects, incompatibility of pharmacokinetic profiles, and some physicochemical properties [2]. Additionally, nanoparticulate systems have contributed to improved efficacy and decreased adverse effects associated with chemotherapy [3]. Therefore, molecular hybrids between 3 α -hydroxymasticadienonic acids and argentinin B (Hib-1) were synthesized, and their cytotoxic activity was evaluated in prostate cancer (PC-3) cells. The results showed that Hib-1 has similar cytotoxic activity to the precursors, but unexpectedly, the solubility of the hybrids decreased. At the same time, the PLGA-500® nanoparticles of the precursor triterpenes were evaluated, and a significant increase in their cytotoxic activity was observed. In summary, the results of the hybrids' biological activity are preliminary, and work is underway to design a nanoparticulate system of these compounds that would allow for realistic observation of their effect on biological activity.

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P360 Antibacterial potential of prenylated polyketide derivatives against multidrug-resistant pathogens

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Multidrug resistance (MDR) is the ability of microorganisms, including bacteria, fungi, and parasites, to survive exposure to multiple antimicrobial agents that are usually effective against them. This resistance poses a significant challenge in treating infections, leading to prolonged illness, increased healthcare costs, and higher mortality rates [1]. Consequently, there is an urgent need for innovative antimicrobial agents to effectively address these resistant pathogens. Building on the evolutionary success of natural products in combating bacterial infections, this study explores the potential of a library of over one hundred plant-derived compounds to address MDR [2]. This library, comprising several structural classes such as terpenes, flavonoids, alkaloids, and phenolics, was evaluated for its antibacterial activity. Initial screening against *Escherichia coli* MG1655 and *Staphylococcus aureus* ATCC 25923 identified several promising compounds exhibiting selective activity (MIC ≤ 8 μ g/mL) against *S. aureus*. Further evaluation confirmed the efficacy of natural and semisynthetic prenylated polyketides against a panel of MDR Gram-positive bacteria. Notably, these compounds exhibited negligible hemolytic activity (<3 %), suggesting a favorable safety profile. Ongoing research focuses on elucidating their mechanism of action using in silico techniques such as Inverse Virtual Screening (IVS) and molecular docking to predict interactions with bacterial targets [3]. These collective findings underscore the potential of prenylated polyketide derivatives as a source of novel antibacterial agents, paving the way for future investigations into their therapeutic application against MDR infections.

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P361 Phytochemical characterization and valorization of *Symphytum officinale* L. roots from continental Croatia

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Comfrey (*Symphytum officinale* L., Boraginaceae) root-based preparations have been used for the management of pain and inflammation associated with musculoskeletal disorders, including conditions affecting joints, bones, and muscles [1]. Metabolomic analyses have shown the complex phytochemical profile of comfrey roots, comprising polysaccharides, allantoin, cinnamic acid derivatives, and pyrrolizidine alkaloids. Furthermore, pharmacological studies have revealed the mechanistic basis of their anti-inflammatory, analgesic, wound-healing,

anti-irritant, and osteo-regenerative activities. Key bioactive constituents, such as allantoin, rosmarinic acid, globoidnans A and B, rabdosiin, have been identified as principal contributors to the therapeutic efficacy of *Symphytum*-derived formulations, particularly in mediating anti-inflammatory and wound-healing responses [2]. The objective of this study was to evaluate the phytochemical composition of comfrey root samples collected from continental Croatia, with the aim of exploring their potential as sources of bioactive compounds for the development of formulations intended for both human and veterinary applications. Root samples were obtained from three distinct geographical regions, Zagreb County, Baranja County, and Požega-Slavonia County. The plant material was extracted using 65 % ethanol through maceration, employing a drug-extract ratio (DER) of 1:10 (w/v). The phytochemical profiling of comfrey extracts was conducted using liquid chromatography-high resolution tandem mass spectrometry (LC-HRMS/MS). All analyzed extracts displayed similar chemical compositions, characterized by the presence of ten phenolic acids (including caffeic acid, rosmarinic acid, globoidnans A and B, rabdosiin), seven pyrrolizidine alkaloids (e.g., intermediate/lycopsamine, acetylintermediate/acetyllycopsamine, symphytine, and their corresponding N-oxides), and three organic acids. Accordingly, this study highlights *S. officinale* roots collected from various regions of Croatia as a valuable source of bioactive compounds, with potential applicability in the development of topical formulations for both human and veterinary use.

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P362 Bioactive triterpene saponins isolated from *Cyclamen hederifolium*

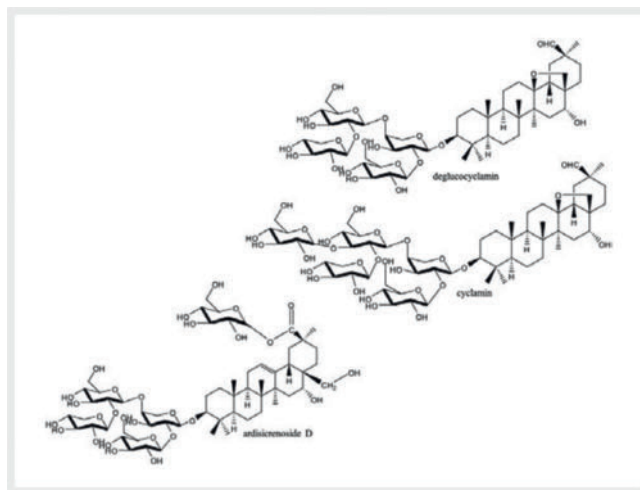
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Cyclamen hederifolium fresh tubers has been used in the Italian traditional medicine in the treatment of hemorrhoids and frostbites. [1] Several studies indicate that *Cyclamen hederifolium* has been used in folk medicine in the treatment of menstrual problems [2], migraine [2], as a sedative, purgative, emmenagogue and anti-helminthic [3] and antitumor agent [4]. Extract of fresh tubers of *Cyclamen hederifolium* were prepared to isolate and identify bioactive constituents.

The tubers were ground, lyophilized, and extracted with methanol at room temperature with the use of an ultrasonic bath. The part of the methanol extract was further fractionated by dissolving in water and then washed with chloroform. Then, water layer was reextracted with *n*-butanol. The butanol extract was fractionated by repeated isocratic column chromatography on silica gel with mixture of CHCl₃/MeOH/H₂O as eluent. This resulted in isolation of triterpene saponins deglucocyclamin, cyclamin and ardisicrenoside D (► Fig. 1). The saponins were identified using 1D and 2D NMR spectroscopy. The triterpene saponin, deglucocyclamine, showed promising results against NSCLC, melanoma, colon, breast, ovarian, kidney, prostate, and CNS cancer cell lines.



► **Fig. 1** Structures of deglucocyclamine, cyclamin, and ardisicrenoside D

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P363 Comparative analysis of *Rosmarinus officinalis* L. essential oil and hydrosol

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Rosmarinus officinalis L. (rosemary) is a well-known herb with strong antioxidant and anti-inflammatory properties [1]. Traditionally used to improve circulation and reduce inflammation, it is widely studied for its potential in treating diseases related to oxidative stress and inflammation. Although most research focuses on its essential oil, studies on both the essential oil and hydrosol remain limited [2, 3].

This study compares the quality and effectiveness of self-extracted and commercial rosemary products. The total phenolic content of a commercially available *R. officinalis* L. essential oil was compared with that of a hydrosol obtained via steam distillation using a Clevenger apparatus.

Plant material was collected from Istanbul during flowering season in 2024. While hydrosol was successfully obtained, essential oil yield was too low for experimental use. Therefore, analyses at this stage were conducted on the hydrosol and a commercial essential oil. The total phenolic content of the hydrosol was determined using the Folin-Ciocalteu method as 27.19 ± 16.01 mg GAE/g, whereas the commercial essential oil showed 57.98 ± 13.23 mg GAE/g, where GAE refers to gallic acid equivalents.

This preliminary investigation aims to contribute to the understanding of the pharmacognostic potential of Turkish rosemary and presents part of the initial findings from a research project developed within the scope of an undergraduate thesis proposal. Further analyses will assess total flavonoid content, DPPH scavenging activity, CUPRAC antioxidant capacity, and anti-inflammatory effects of both product types.

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P364 GC/MS analysis of alkaloidal extracts from *Hypecoum procumbens* L. subsp. *atropunctatum* Å. E. Dahl

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Plants belonging to the genus *Hypecoum* Tourn. ex L. (Papaveraceae) are widely distributed from the Mediterranean region to Central Asia and Northern China [1]. Among the *Hypecoum* species found in Turkey, *Hypecoum procumbens* L. is represented by two subspecies [2]. Phytochemical characterization of *Hypecoum* species reveals that the main active constituents are isoquinoline alkaloids with various chemical classes and biological activities which could be considered as significant chemotaxonomic markers. Among these protopine is the main alkaloid isolated from the species of this genus and it has been shown to possess important biological properties, including antimicrobial, anticancer and anti-inflammatory activities [1, 3]. In the present study, the alkaloidal profiles of the extracts of plant specimens collected from four different localities in Western Turkey, were analyzed by GC/MS by using a previously described method [4]. As a result, isoquinoline alkaloids belonging to different chemical groups were detected in variable amounts. Protopine, a representative alkaloid of protopines, was the predominant alkaloid in all of the extracts detected within the range of 69.39–96.2%. Leptopinine (secoberbine type) was also found in significant amounts (3.72–23.03%) in the extracts. Dihydrosanguinarine (benzophenanthridine type) and a simple isoquinoline alkaloid oxyhydrastinine were detected up to 6.51% and 1.07%, respectively. As evident from the literature [1, 3], the phytochemical investigation of *Hypecoum* species is important as the structural types of the identified isoquinoline alkaloids can be used to determine the relationships between members of this genus and other genera in the Papaveraceae family.

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P365 Preliminary study on the aerial parts and roots of *Limbaria crithmoides* L., a Mediterranean halophyte

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Limbaria crithmoides L. is a common but still largely uninvestigated edible halophyte of the Asteraceae family, spontaneous in the Mediterranean region. In the Middle East, the decoction obtained from its roots is regarded as a remedy for gastrointestinal and hepatic ailments [1].

Given the increasing scientific interest in recent years due to halophytes' favourable adaptation to the changing climate, the purpose of this work was to investigate the phytochemical composition of both aerial (LICA) and underground (LICR) parts of this species. Additionally, the bioactivity and bioaccessibility of the phytocomplex was assessed.

Extracts obtained from the dried plant materials with different solvents and preparations were screened through colorimetric assays to highlight the main chemical differences. As expected, polysaccharides represented a high percentage of the plant material, reaching 35.7 ± 0.51 % (w/w as saccharose equivalents) in LICA EtOH 25 % v/v, while triterpenes were more abundant in LICA decoction (3.60 ± 0.02 % w/w as β-sitosterol equivalents). Total phenols peaked in LICR EtOH 75 % v/v, 4.10 ± 0.05 % (w/w as gallic acid equivalents), in agreement with the most potent DPPH-radical scavenging activity measured for this extract (IC₅₀ = 0.125 ± 0.003 mg/mL). Moreover, the HPLC-DAD analyses highlighted abundant hydroxycinnamic derivatives, such as chlorogenic acid, in both plant parts. Thus, an *in-vitro* simulated digestion model was set up, allowing us to appreciate how the total hydroxycinnamic derivatives, despite some modifications, could be recovered after the process both in LICA and LICR (Table). Therefore, this preliminary study paves the way for future bioactivity and phytochemical studies on *L. crithmoides* L.

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P366 Src tyrosine kinase inhibitory and antioxidant activity of *Stevia rebaudiana* Bertoni

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Stevia rebaudiana Bertoni (Stevia) is a perennial herb of the Asteraceae family, a well-known source of the natural sweetener steviol glycosides. Isolation of the sweet compounds from Stevia generates large amounts of waste, which could be a rich source of many bioactive compounds. Previous studies have shown promising health benefits of Stevia against diabetes, obesity, hypertension, cancer, dental caries and microbial infections [1, 2]. Therefore, the aim of our study was to investigate the polyphenolic composition of the ethanolic extract of Stevia leaves and, for the first time, its ability to inhibit Src tyrosine kinase activity and scavenge NO free radicals. The total content of phenolic acid and flavonoids (5.06% and 1.36%, respectively) in Stevia leaves was determined spectrophotometrically. Eight hydroxycinnamic derivatives and thirteen flavonoids were identified in the ethanolic extract by LC-ESI-MS/MS. Caffeoylquinic acids were found to be the predominant constituents (7.62–55.21 mg/g). The ethanolic extract inhibited Src tyrosine kinase activity in the time-resolved fluorescence resonance energy transfer (TR-FRET) assay (IC₅₀ = 213 µg/mL). It also showed a strong ability to scavenge NO radicals (IC₅₀ = 67.58 µg/mL). Our study suggests that Stevia may be beneficial in cancer and other inflammation-related diseases, with chlorogenic acid and its derivatives making a substantial contribution.

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P367 Irish bogland plants: From their role in peat formation to a rich source of bioactive compounds

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The tradition of cutting turf, or harvesting peat, is deeply embedded in Irish history, with peatlands covering approximately 16% of the country. For centuries, this practice provided essential heating for homes and served as a source of income for families, enabling strong community connections as families and neighbors worked together in the summer. By the 21st century, legal restrictions emerged, prompting a shift towards more sustainable turf cutting practices. Irish bogland is home to a unique array of plant species that are particularly well-adapted to the wet, acidic conditions found in these ecosystems. Examples include sphagnum mosses, heather (*Erica* spp.), cotton grass (*Eriophorum* spp.), tormentil (*Potentilla erecta*), bog rosemary (*Andromeda polifolia*), the insectivorous sundews (*Drosera* spp.), lichens, liverworts, bog myrtle (*Myrica gale*) and Devil's bit scabies (*Succisa pratensis*)/silver birch (*Betula pubescens*) at the edge of bogs [1]. These contain numerous classes of bioactive secondary metabolites which range from flavonoid glycosides, diaryl nonanoids, caffeoyl quinic acids, coumaroyl glycosides, pentacyclic triterpenes, naphthoquinones to hydrolysable tannins. The NatPro team has developed optimal secondary metabolite extraction procedures for a selection of these plants, performed both qualitative and quantitative analysis and evaluated their biological activity. Our assays have measured the anti-inflammatory activity, insect repellency properties and their effects in cellular migration, invasion and *ex vivo* angiogenesis models.

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P368 Anti-hyperglycemic effect of the roots of *Capparis erythrocarpus* in mice

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Diabetes mellitus (DM) is a chronic metabolic disease characterized by persistent hyperglycemia, affecting millions of people globally [1]. Due to undesirable side effects of current antidiabetic drugs [2], there is still a need for safer alternatives. The roots of *Capparis erythrocarpus* (CE) Isert (Capparaceae) are used in the management of DM in many parts of Africa, including Tanzania [3, 4]. This study aimed to investigate the anti-hyperglycemic effects of CE and to isolate bioactive constituents from CE roots. The anti-hyperglycemic effect of the crude ethanolic extract (CEE) and dichloromethane (DCM), ethyl acetate and ethanol fractions (dose: 500 mg/kg body weight (b.w.)) was evaluated *in vivo* in healthy albino mice using the oral glucose tolerance test. Chlorpropamide (100 mg/kg b.w.) and 1% carboxymethylcellulose served as positive and negative control, respectively. Phytochemical screening was conducted using

chemical reactions for major phytochemical classes and ultra-high performance liquid chromatography (UPLC) – high resolution mass spectrometry (HRMS). Isolation of compounds from the most active fraction was performed using flash chromatography and semi-preparative HPLC with Diode Array Detector (DAD) and MS detection. Structure elucidation of isolated compounds was achieved using Nuclear Magnetic Resonance (NMR) spectroscopy. The CEE and DCM fraction exhibited a significant anti-hyperglycemic effect compared to the negative control ($p < 0.05$), and phytochemical analysis revealed the presence of steroids, alkaloids and saponins. Our results indicate the potential antidiabetic activity of CE, while further studies are ongoing to determine the identity and activity of its isolated constituents.

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P369 Phytochemical variability in *Drosera rotundifolia* and its relevance for medicinal plant cultivation

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Round-leaved Sundew (*D. rotundifolia* L., Droseraceae), a carnivorous medicinal plant traditionally used to treat respiratory tract infections, has the potential to address two challenges: providing economic use for renatured peatlands and combating multidrug-resistant bacteria through its biofilm-inhibiting properties. The bioactive compounds responsible, flavonoids and naphthoquinones, have already been identified [1]. A next step is to ensure a consistently high quality of plant raw material, e.g. by identifying plants with an optimal chemotype for cultivation.

To determine the concentrations of active compounds required for biofilm inhibition, sundew samples were collected from 67 populations across 12 European countries. Extracts were tested for their ability to inhibit biofilm formation in multidrug-resistant Gram-negative (MRGN) *Escherichia coli* using a macrocolony assay. High-performance liquid chromatography with diode-array detection (HPLC-DAD) was employed to quantify active compound contents. To investigate genetic and environmental influences on phytochemical composition, a subset of collected plants was cultivated under standardized conditions in a Common Garden Experiment (CGE).

Preliminary results indicate that the geographic origin of wild sundew affects biofilm inhibition. Additionally, phytochemical concentrations varied more between different sampling areas (for naphthochinones 4.5 times higher than the lowest concentration) than within a single sundew population. This variability could result from genetic differentiation, explaining some differences in phytochemical composition of sundew from the CGE, or site-specific environmental conditions that favor the production of certain compounds. To better understand these influences, genetic analyses are planned that will ultimately lead to the identification of optimal plant genotypes or cultivation conditions for the medicinal use of sundew.

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P370 7 α -acetoxy-6 β -hydroxyroyleanone isolated from *Plectranthus hadiensis* as anti-glioblastoma drug candidate

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The plant genus *Plectranthus* is known to be rich in abietane royleanone-type diterpenes with reported antitumor activity against various cancers. One such compound, 7 α -acetoxy-6 β -hydroxyroyleanone (Roy), can be isolated from *P. hadiensis*. Among different cancer types, glioblastoma (GB) is the most common and aggressive brain tumor, highlighting the urgent need for new therapeutic options due to resistance and adverse effects associated with current treatments [1, 2].

This study aimed to perform the bioguided isolation and quantification of Roy from the acetonic extracts of *P. hadiensis* leaves and stems, as well as to investigate its *in vitro* anti-glioblastoma activity. Additionally, the biodistribution, mechanism of action, and formulation of self-assembled nanoparticles (NPs) incorporating Roy were evaluated [4].

Quantitative analysis by HPLC-DAD revealed that Roy was 13.4 times more abundant in the acetonic extract of leaves compared to stems [2, 3]. Biodistribution studies demonstrated intracellular accumulation of Roy, while mechanistic investigations showed that it induced intrinsic apoptosis and cell cycle arrest in the G2/M phase. Notably, the concentrations of Roy required to achieve significant inhibitory effects were 6–9 times lower than those of TMZ, the standard chemotherapeutic agent for GB [3, 5].

These findings highlight Roy as a promising antitumor compound, with potential for formulation as self-assembled oleic acid NPs to enhance its passage through the blood-brain barrier, improving therapeutic outcomes in glioblastoma treatment.

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P371 Citrus pomace extracts preserve intestinal barrier integrity and exhibit antioxidant, anti-inflammatory and wound-healing activities in Caco-2 cells

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Leaky gut is characterized by increased intestinal permeability due to disrupted tight junctions, allowing harmful molecules to pass through and activate the immune system. Persistent permeability can lead to chronic inflammation, damaging the intestinal wall and contributing to Inflammatory Bowel Diseases (IBD) [1].

This study, aligned with circular economy principles, investigates the effects of food-grade extracts from orange (OE) and lemon pomace (LE) on lipopolysaccharide (LPS)-induced inflammation and wound healing in Caco-2 cells.

Phytochemical characterization of OE and LE was conducted via LC-DAD-ESI-MS/MS, followed by HPLC-DAD titration of key compounds: hesperidin and narirutin for OE, hesperidin and eriocitrin for LE. Intestinal bioaccessibility, antioxidant, and anti-inflammatory activities were evaluated through *in vitro* simulated gastrointestinal digestion and treatment of LPS-stimulated Caco-2 monolayers.

The analysis focused on structural proteins related to tight junctions (ZO-1, Claudin-1, Occludin), transepithelial electrical resistance (TEER), reactive oxygen species (ROS) levels, and the expression of antioxidant (CAT, NRF2, SOD2) and inflammatory (IL-1 β , IL-6, TNF- α , IL-8) markers. Wound-healing activity was also assessed using hyaluronic acid as a positive control.

Both OE and LE digests demonstrated protective effects against LPS-induced intestinal barrier damage. They effectively reduced oxidative stress, inflammatory responses, and promoted wound healing.

In conclusion, although further studies are required to substantiate this hypothesis, including the use of an *in vitro* co-culture system involving mucus-secreting goblet cells and immune cells, which is currently underway, these findings highlight OE and LE as promising candidates for further preclinical and clinical studies in IBD management.

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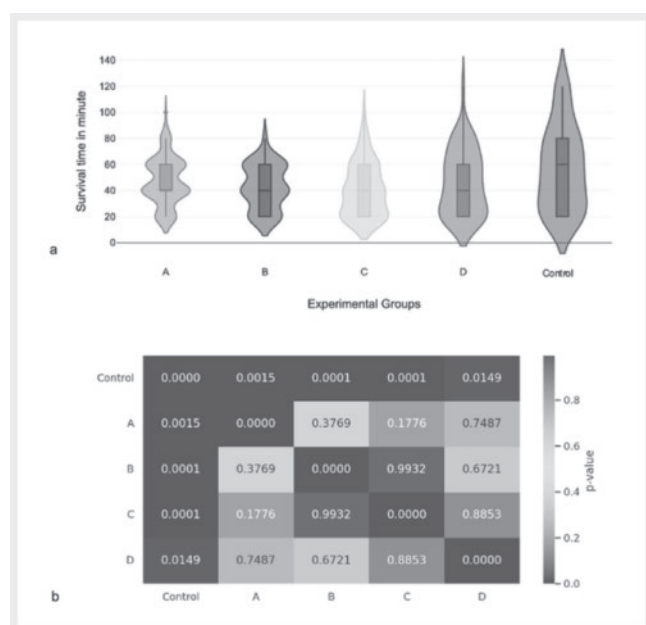
P372 Thermotolerance-based screening of *Doronicum hakkaricum* extracts in *C. elegans*

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Doronicum hakkiaricum is an endemic species from the Asteraceae family in Türkiye that remains largely unexplored in terms of pharmacological properties, despite the traditional medicinal use of its genus [1, 2]. This study aimed to evaluate the thermotolerance-modulating effects of *D. hakkiaricum* ethanolic extracts in *Caenorhabditis elegans*. Wild-type N2 *C. elegans* were exposed to four extract conditions (100% and 50% ethanol; each 100 µg/mL and 1000 µg/mL) under thermal stress at 35 °C [3]. Survival was recorded at 20-minute intervals. Kaplan-Meier survival analysis, log-rank test, and Fisher's exact test with Bonferroni correction were used for statistical comparisons. All treatment groups showed a significant reduction in lifespan compared to controls. The most prominent decrease was observed with the 50% ethanolic extract at 1000 µg/mL (mean lifespan: 40.46 ± 2.13 min), whereas the control group exhibited the highest thermotolerance (57.18 ± 3.58 min). A non-linear dose-response relationship was observed, indicating that higher concentrations did not consistently lead to increased toxicity. Statistical analysis ($p < 0.05$) and heatmap visualization confirmed solvent- and dose-dependent activity (► Fig. 1). These results suggest selective extraction of bioactive compounds depending on solvent composition and raise the possibility of a potential anthelmintic effect, considering *C. elegans* as a nematode model. To our knowledge, this is the first report on the biological activity of *D. hakkiaricum* in *C. elegans*. The authors declare no conflict of interest.



► **Fig. 1** (a) Violin and boxplot showing the survival time distribution of *Caenorhabditis elegans* under heat stress (35 °C) following treatment with *Doronicum hakkiaricum* extracts. Control and four treatment groups (A: 100% EtOH extract, 1000 µg/mL; B: 100% EtOH extract, 100 µg/mL; C: 50% EtOH extract, 1000 µg/mL; D: 50% EtOH extract, 100 µg/mL) are compared. (b) Heatmap of pairwise comparisons using the log-rank (Mantel-Cox) test showing statistical differences in survival among experimental groups. The intensity of color reflects the level of significance, with darker tones indicating stronger divergence. All treatments significantly reduced thermotolerance compared to the control group.

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P373 Enhanced antioxidant activity of carvacrol via nanoformulation: A promising strategy for cosmetic industry

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Carvacrol (Car) is a monoterpene compound found in *Oregano* species and has numerous biological activities such as antioxidant effect. However, direct use of the Car molecule is difficult due to its pungent odor, easy oxidation and irritating structure. Nowadays, loading into polymeric structures in the nanoparticles is becoming widespread to reduce such as side effects [1]. Our previous study evaluated collagenase inhibitory activity by encapsulating Car into polycaprolactone (PCL) nanoparticles (NPs). In this study, the antioxidant activity of Car-PCL-NPs obtained using the same encapsulation system was examined and its effectiveness was evaluated in comparison with free Car [2]. It was predicted that the antioxidant activity of Car, whose irritating effect and strong odor were already eliminated by encapsulation, would also increase in this form.

In the study, ABTS, DPPH, CUPRAC and metal chelating antioxidant tests were performed on free Car and Car-PCL-NPs. As a result, free Car showed antioxidant effect in all of them, but it was observed that Car-PCL-NPs were active only in ABTS and CUPRAC tests. This activity is approximately 10 times higher in the ABTS test and 8 times higher in the CUPRAC test compared to free Car. According to the results, it was determined that the activity of the Car loaded into NPs increased significantly. Also, volatility and irritating properties were eliminated, making Car easier to use. Thus, it was predicted that Car-PCL-NPs could be suitable candidates for use in the cosmetic industry as more stable and bioavailable active structures for anti-aging, skin brightening topical formulations.

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P374 Cheminformatics identification of phenolics as modulators of penicillin binding protein (PBP) 5 of *Enterococcus faecium* towards interventional antibacterial therapy

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Penicillin-binding protein 5 (PBP5) of *Enterococcus faecium* is a high molecular mass protein crucial for the synthesis of peptidoglycan, a key component of the bacterial cell wall. This protein has garnered significant attention as a major therapeutic target due to its reported ability to reduce affinity for commonly prescribed β -lactams, leading to persistent antibiotic resistance. Consequently, bioprospection for new chemical entities with novel mechanisms of action against PBP5 of *E. faecium* remains one of the viable strategies to address this persistent menace. This study involved bioprospection of 1225 phenolics against the binding domain of PBP5, and the top five phenolics with best docking scores, acceptable pharmacokinetic attributes, and synthetic traits were

further thermodynamically evaluated via a 200-ns molecular dynamics simulation period. Ph92237944, with the least docking score also showed a better binding affinity (-19.7 kcal/mol) for PBP5 relative to the reference standard [ampicillin (-12.31 kcal/mol)]. In addition, post-dynamic evaluation showed Ph92237944 exhibited strong affinity and stability within the binding region of PBP5, as evidenced by stable hydrogen bonds, Van der Waals forces, and hydrophobic interactions. These interactions contributed significantly to the overall stability and affinity of the Ph92237944-PBP5 complex. Therefore, based on this finding, Ph92237944 has the potential to interact with and modulate the activity of low-affinity PBP5 of *E. faecium*, highlighting its potential for further investigation as a prospective antibacterial agent, pending experimental validation [1–3].

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P375 NMR HetCA, HPTLC-DPPH • bioautography and LC-MS methodologies for the direct identification of bioactive compounds from *Juglans regia* L. and *Sambucus nigra* L.

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The present study was carried out within the European project EthnoHERBS in which, *Juglans regia* L. and *Sambucus nigra* L. species were selected for further investigation as there were extensive references in ethnopharmacological studies for their use in the treatment of skin ailments [1, 2]. Initially, LC-MS dereplication facilitated the annotation of 61 compounds from the methanolic extract of *J. regia* (flavonoids and naphthoquinone derivatives), while from the hydroalcoholic extract of *S. nigra* were annotated 36 metabolites (phenolic derivatives and flavonoids). In parallel, both extracts were fractionated by Fast Centrifugal Partition Chromatography (FCPC) and then, High Performance Thin Layer Chromatography (HPTLC) was performed to investigate their chemical content, while simultaneously the antioxidant capacity of their ingredients was evaluated using HPTLC bioautography (HPTLC-DPPH •). In addition, NMR spectra of the fractions were acquired while their DPPH free radical scavenging capacity and inhibitory activity against collagenase enzyme was evaluated *in vitro* and the Heterocovariance Approach (HetCA) was employed in MATLAB. From the correlation of the spectral data and results of bioactivity assays, high correlation in HetCA plots (pseudospectra) indicated responsible for the activities quercetin derivatives and 1,4,8-trihydroxynaphthalene 1-O-glucopyranoside, in the case of *J. regia*, while for the hydroalcoholic extract of *S. nigra* the caffeoylquinic acid and quercetin derivatives were directly identified as active components. In conclusion, HetCA approach and

HPTLC bioautography, facilitated the identification of bioactive metabolites prior to their isolation, accelerated the experimental process and reduced the cost of the analysis required by conventional approaches for the study of complex natural preparations.

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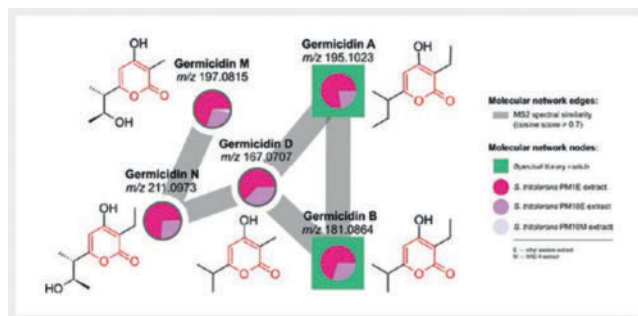
P376 Computational mass spectrometry-guided discovery of bioactive compounds from the underexplored, polyextremotolerant, marine-derived actinobacterium *Streptomyces tritolerans*

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The phylum *Actinomycetota* is remarkably vast, encompassing species with extensive biological and chemical diversity, such as those within the genus *Streptomyces*, renowned for producing bioactive compounds and explored for their biotechnological potential. Almost two decades ago, the species *Streptomyces tritolerans* was discovered [1], yet it remains largely underexplored, both biologically and chemically, especially considering its specialized metabolites. In continuation of our research of *Streptomyces* spp. [2], we present the investigation of extracellular chemical profile of three *S. tritolerans* extracts derived from culture supernatants of two strains, guided by a computational mass spectrometry. The extracts were obtained via liquid-liquid and sorbent-assisted extraction and subsequently analyzed using UHPLC-HRMS. The MS/MS data from the crude extracts were used to: (a) preliminarily identify known bioactive compounds, (b) annotate their related unknown features, and (c) assist in planning their isolation strategy. For feature dereplication, we employed spectral library matching via the GNPS platform, *in silico* fragmentation tree analysis, and chemical class prediction. We then constructed molecular networks to annotate the related unknown features and design their purification strategy. This integrated approach led to the discovery, isolation, and structural elucidation—based on LC/MS and NMR—of five α -pyrone analogs: germicidins A, B, D, M, and N, for the first time in *S. tritolerans* (► Fig. 1). Although several of these compounds demonstrate antimicrobial and antioxidant capabilities, some evidence suggests their potential for simple and effective conversion into α -pyridones, known for their broad spectrum of bioactivities.



► Fig. 1 Molecular network of α -pyrone analogs (germicidins), isolated and structurally elucidated from the extracts of *Streptomyces tritolerans*

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P377 Integrating Box-Behnken experimental design with metabolomics to navigate microbial metabolite production

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The suppression of numerous secondary metabolite gene clusters under standard laboratory culture conditions and the frequent re-isolation of known compounds represent critical challenges in the discovery of natural therapeutic leads. Elicitation methods to activate secondary metabolite gene clusters and dereplication strategies to avoid isolation of known compounds have been used independently, but their integration offers new possibilities for directing microbial secondary metabolism. In this work, we developed a “culture condition-based metabolomics” approach by combining Box-Behnken experimental design [1] with LC-MS/MS-based metabolomics to systematically explore and navigate secondary metabolite production. *Streptomyces cacaoi* was cultivated under 71 different fermentation conditions established using Box-Behnken designs with glycerol, peptone, CaCO₃, FeCl₃, MgCl₂, KNO₃, Li₂CO₃, DMSO, temperature, and seawater as independent variables. Ethyl acetate extracts were prepared, analyzed via LC-HRMS/MS, and processed with MZmine [2]. Metabolites were annotated using feature-based molecular networking [3] and SIRIUS-based tools [4], with key compounds isolated and confirmed by NMR [5]. By treating each detected feature's relative abundance as a response variable within the statistical design, we identified specific relationships between fermentation conditions and metabolite production. Statistical analysis revealed that high temperatures, high peptone concentrations, and MgCl₂ induced various polyketide and terpenoid metabolites. Specifically, high temperatures induced non-glycosylated K-41A derivatives while suppressing 2'-deoxyadenosine and marineosins. FeCl₃ supplementation enhanced phenolic acids while inhibiting ferrioxamine production. KNO₃ suppressed polyketide pathways but induced ectoine production. This integrated approach systematically identified conditions that directed secondary metabolism toward the desired biosynthetic pathways, facilitating the discovery of novel microbial natural products.

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P378 Investigation of *Hypericum* species of greek, bulgarian and serbian flora. NMR-Heterocovariance (NMR-HetCA) chemometrics approach for the identification of bioactive compounds of *H. empetrifolium*

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In EthnoHERBS EU project, following thorough ethnopharmacological research in the Balkan Peninsula, the Hypericaceae plant family showed substantial potential for discovering dermatologically active therapeutic agents [1]. This study aimed to evaluate the phytochemical and biological properties of selected Hypericaceae species (*Hypericum empetrifolium*, *H. maculatum*, *H. perforatum*, *H. barbatum*, *H. triquetrifolium*), traditionally used against various skin disorders. The dichloromethanic, methanolic, and hydromethanolic (50:50, v/v) extracts were analysed via High-Performance Thin-Layer Chromatography (HPTLC), while total phenolic (TPC) and total flavonoid (TFC) content were quantified using the Folin-Ciocalteu and aluminum chloride methods, respectively. Antioxidant activity was assessed using the DPPH assay (200 µg/mL), and inhibitory effects on collagenase and tyrosinase were also evaluated. The methanolic extract of *H. empetrifolium* aerial parts showed notable antioxidant and anti-collagenase activity and was selected for further fractionation using Centrifugal Partition Chromatography (CPC). Fractions were evaluated for their free radical scavenging using the HPTLC-DPPH bioautography method. Moreover, the methanolic extract showed significant *in vitro* collagenase inhibition, an enzyme relevant to wound healing and anti-aging, that comes in agreement with the traditional use of the plant species in Southeastern Europe to speed the healing process of wounds and scabs. To identify bioactive metabolites prior to isolation, the NMR-Heterocovariance (NMR-HetCA) chemometrics approach was employed, correlating NMR spectra with biological data using SHY and STOCSY tools within the MATLAB environment. This method proved effective for detecting minor bioactive metabolites. In parallel, LC-MS dereplication led to the identification of 40 compounds, including flavonoids, phenolic acids, catechin derivatives, and their glucosides.

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P379 Whole-cell biotransformation of parthenolide by fungal strains

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Biotransformation is increasingly recognized as a green, cost-effective and efficient strategy for generating chemodiversity. Previous work in our laboratory demonstrated that fungal secretomes—enzyme mixtures secreted by fungi can efficiently convert simple starting materials into complex molecules [1–3]. However, this approach is limited by the loss of intracellular enzymes and reduced activity of enzymes that rely on cofactors. To overcome these challenges, we developed a high-throughput whole-cell microbiotransformation platform based on fungal cultures grown in 96-well plates [4]. This method enables rapid screening of biotransformation capabilities across multiple fungal strains while minimizing substrate consumption. The platform leverages recent advances in UHPLC-HRMS/MS to facilitate compound identification and gain structural insight. This methodology was applied to the biotransformation of parthenolide, a sesquiterpene lactone from *Tanacetum parthenium*, known for its potent anti-inflammatory and anti-cancer properties [5]. Two strains of *Botrytis* and one of *Sclerotinia* from the Agroscope mycotheca were selected for their ability to generate uncommon structural features. Biotransformations were scaled up to liter-scale cultures, with parthenolide administered in incremental doses to enhance yield and reduce potential substrate inhibition. Reaction monitoring was conducted via UHPLC-DAD-CAD-MS. Purification of the products was achieved using high-resolution semi-preparative HPLC, combining geometric transfer and dry load injection. Structural elucidation was performed through comprehensive NMR and HRMS analyses. Using this workflow, we successfully synthesized a series of parthenolide derivatives, currently under evaluation as Wnt pathways inhibitors in triple-negative breast cancer cells. This sustainable approach provides a promising avenue for generating novel chemical scaffolds with potential bioactivities.

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P380 A data-driven machine learning platform for natural products discovery from the microbiome using integrative multiomics

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The human microbiome is a dynamic ecosystem whose composition is increasingly linked to health and disease. While sequencing technologies have expanded our understanding of microbial diversity, the next frontier lies in decoding the evolved mediators of microbial function and their host interactions. Small molecules encoded by the microbiome represent key communication chemistries, yet functional prediction from the vast gene pool remains a major challenge. Although landmark examples, such as anti-MRSA agents from nasal commensals, highlight therapeutic potential, most discoveries have occurred by chance. Here, we present a machine learning platform that integrates metagenomic, transcriptomic, and proteomic data to systematically infer gene function and uncover drug-like molecules. We designed and generated a Middle Eastern human microbiome dataset and used it to reveal thousands of novel genes with no known homologs. The workflow identified antagonistic relationships between these genes and human pathogens, including *Listeria*, *Shigella*, and *E. coli*. For experimental validation, we synthesized five gene products predicted to have narrow-spectrum activity against resistant strains such as MRSA. In vitro assays confirmed antimicrobial efficacy with minimal cytotoxicity on human cell lines, validating the predictive framework. By enabling targeted discovery of bioactive small molecules, this work provides a scalable engine for natural products drug discovery. The platform holds transformative value for both basic and translational microbiome research. Additionally, this research addresses critical equity gaps by profiling underrepresented populations and marks one of the first pioneering efforts to build a microbiome database in the Middle East [1–5].

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P381 Silicristin-mediated lethality towards *Staphylococcus aureus*: Evidence from oxidative stress contribution and molecular dynamics simulation

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Beyond their primary role in modulating penicillin-binding proteins (PBPs), β -lactams have been linked to reactive oxygen species (ROS) generation in bacteria [1]. This effect on bacterial macromolecules presents a promising avenue for novel therapeutic development. This study explores the modulatory effect of silicristin on *Staphylococcus aureus* PBP2a and its potential to induce oxidative stress, both *in silico* and *in vitro*. Molecular dynamics (MD) simulations revealed that silicristin exhibited significant affinity (–25.61 kcal/mol) towards PBP2a binding domain compared to amoxicillin (–21.54 kcal/mol) over a 120-ns exposure period. This observation was further supported by the Density Functional Theory (DFT) B3LYP/6-31G+(dp) analysis, where silicristin demonstrated higher reactivity than cefotaxime, aligning with the thermodynamic stability and compactness of the resulting complexes. Further investigation through *in vitro* treatment of *S. aureus* ATCC 43300 with silicristin suggested an oxidative stress-mediated bactericidal effect, indicative of a greater impact through the Fenton reaction compared to the observation with amoxicillin. Notably, protein carbonyl and malondialdehyde levels were significantly elevated following silicristin treatment.

ristin treatment. Scanning electron microscopy further revealed extensive damage to the bacterial cell wall, with more pronounced blisters, eruptions, and sores in silicristin-treated cells than in both untreated and amoxicillin-treated cells. Additionally, oxidative stress triggered an SOS response, upregulating *SordA* and *SoxR*, with the strongest effect observed in silicristin-treated cells. These findings suggest that silicristin enhances redox stress in *S. aureus* and may be promising in designing novel β -lactam-based therapeutics against *S. aureus*-mediated infections.

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P382 Chemical profiling and antibacterial evaluation of essential oils from *Pistacia falcata* Becc. ex-Martelli

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The chemical composition of essential oils extracted from various parts of *Pistacia falcata* Becc. ex-Martelli including inflorescences, fruiting inflorescences, roots, and leaves was analysed. The essential oils revealed a dominance of monoterpene hydrocarbons, ranging from 74.6% to 94.5%. The dominant monoterpene hydrocarbon was α -pinene in the roots (80.4%), followed by myrcene in the leaves (45.1%). Oxygenated monoterpenes ranged from 1.0% to 9.0%, with bornyl acetate being the most prominent (4.8%) in the inflorescence. Sesquiterpene hydrocarbons were present in amounts ranging from 0.0% to 7.1%, with bicyclogermacrene being the major constituent (2.4%) in the inflorescence. Oxygenated sesquiterpenes ranged from 0.1% to 7.2%, with (*E*)-nerolidol being the most abundant in the roots. The antibacterial activity of these oils was assessed against *Staphylococcus aureus* NCTC 6571, *Escherichia coli* MG1655, and *Pseudomonas aeruginosa* PAO1. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays revealed moderate activity of the inflorescence and fruiting inflorescence oils against *S. aureus* (MIC and MBC = 5 mg/mL). Leaf oil showed moderate activity (MIC = 10 mg/mL), while root oil was inactive (MIC > 20 mg/mL). No inhibition was observed against *E. coli* or *P. aeruginosa*, although pigment alterations in *P. aeruginosa* were noted at 5–10 mg/mL. Growth kinetics confirmed sustained inhibition by the inflorescence oil over 24 hours at 5 mg/mL. However, biofilm formation by *P. aeruginosa* was not significantly affected. These results highlight the moderate antibacterial potential of *P. falcata* floral essential oils, particularly against Gram-positive bacteria [1].

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P383 Synergistic effects of essential oils and their components with antibiotics against nosocomial pathogens

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The spread of nosocomial infections contribute to significant morbidity, mortality, and financial burden on patients, families, and healthcare systems. The application of different devices (e.g. catheters, implanted devices) are risk factors in the invasion of nosocomial pathogens [1, 2]. The phenomenon of antibiotic resistance and the emergence of multi-resistant pathogens make it vital to investigate new active substances that may even show synergistic effects with antibiotics.

Essential oils (EOs) and their components are known to have antibacterial activity. Our study aimed to investigate the interaction of antibiotics and the EO of lavender, thyme, goldenrod, wintergreen, tea tree, clove, ceylon cinnamon bark, fennel and peppermint or their components. *Escherichia coli*, *Klebsiella pneumoniae*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, *Streptococcus pneumoniae* and vancomycin-resistant *Enterococcus faecalis* (VREF) were included in our study. The chemical composition of the EOs was determined by GC-MS. After establishing the antibiotic resistance profile of the pathogens, minimum inhibitory concentrations (MICs) were determined using microdilution method. Checkerboard titration method was used for combination of antibiotics and/EOs or their components. The fractional inhibitory concentration index (FICI) was calculated based on MIC values. Synergistic effect was demonstrated in several EO-antibiotic combinations (e.g.: MRSA: vancomycin-thyme, vancomycin-fennel, *P. aeruginosa*: gentamicin-lavender and linalool, gentamicin-wintergreen and methyl salicylate, VREF: ampicillin-goldenrod, *E. coli*: ceftriaxone-goldenrod). In these cases, the main components of the EOs also showed synergistic effect with antibiotics (FICI less than 0.5).

Our *in vitro* results have supported the synergistic effect between EOs or their components and antibiotics, therefore, lower antibiotic concentrations can be applied.

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P384 Comparative analysis of essential oils and hydrolates from aniseed, coriander and fennel fruits

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Plants from the Apiaceae family are well-known for their aromatic, nutraceutical, and medicinal properties [1]. They are often used as spices, dietary and functional food supplements. These properties are attributed to essential oils (EOs), which possess a distinctive aroma and promising biological potential [2, 3]. However, the chemical composition and biological activity of the hydrolates (HYs) from these plants remain underexplored. Since some compounds from the EO are transferred to the HYs, they also display aromatic properties and potential biological activity. This study provides a comprehensive analysis of the volatile compounds from the fruits of aniseed (*Pimpinella anisum*), cori-

ander (*Coriandrum sativum*), and fennel (*Foeniculum vulgare*). Essential oils and hydrolates were extracted via steamdistillation under industrial conditions and analysed using gas chromatography-mass spectrometry. Sixty-five components, primarily oxygenated monoterpenes, were identified, highlighting trans-anethole, linalool, and fenchone as major constituents in the respective EOs. Comparative analysis revealed significant differences between the EOs and HYs, with the latter exhibiting distinctive compositions, such as higher proportions of p-anisaldehyde in aniseed and fenchone in fennel. These findings underline the aromatic and potential biological activities of both EOs and HYs, offering insights into their applications in the food, fragrance, and therapeutic industries. This comprehensive characterization enhances the understanding of these Apiaceae species and supports the broader utilization of their HYs as sustainable and functional products in addition to their EOs.

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P385 In vitro evaluation of essential oils as multi-target agents: a natural solution for chronic wound infections

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Chronic wounds, characterized by delayed healing, primarily due to infections with multi-resistant microorganisms and biofilm formation, urge the development of effective strategies [1], such as essential oils (EOs) [2, 3]. We explored the potential of three commercially available EOs (*Citrus limon*, *C. clementina*, and *C. citratus*) for innovative topical treatments targeting infected wounds. The EOs were characterized by GC-MS and then evaluated for: antimicrobial activity against reference and wound infections pathogens (disk diffusion and microdilution assays); antibiofilm activity (violet crystal microtiter), antioxidant activity (DPPH, CUPRAC, FRAP assays) and hemocompatibility (ISO 10993-4:2017 standard assay).

Chemical profiles: *C. limon* (limonene, β-pinene, γ-terpinene); *C. clementina* (limonene); *C. citratus* (geraniol, neral, geranial). Biological activities: i) broad-spectrum antimicrobial activity (minimal inhibitory concentrations of 26-3.25 mg/mL, ii) high efficacy against *Serratia marcescens*, *Staphylococcus aureus*, and *Escherichia coli*; iii) *Candida albicans* and *C. parapsilosis* adherence inhibited by *C. citratus*, being the most effective against clinical isolates; *C. clementina* has significantly reduced *S. aureus* ATCC 25923 mature biofilm and *C. citratus* the *S. marcescens* biofilms iv) *C. citratus* EO showed the most potent antioxidant potential; v) *C. limon* EO exhibited the lowest cytotoxicity. The EOs of *C. limon*, *C. clementina*, and *C. citratus* exhibited notable antimicrobial, antibiofilm, and antioxidant properties, with *C. citratus* stands out for its superior antioxidant performance. All EOs, especially *C. limon*, demonstrated hemocom-

patibility. These findings underscore their potential for inclusion in antimicrobial and antioxidant formulations aimed at managing chronic wound infections and combating biofilm-associated challenges.

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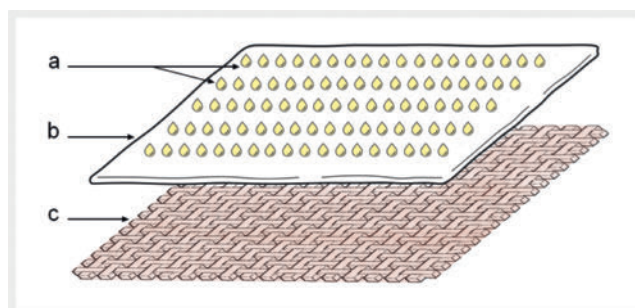
P386 Design of a biobased wound dressing against pressure ulcers

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Pressure ulcers are a major public health issue that the current therapeutic framework is struggling to address [1]. This complex condition combines tissue irritation, inflammation and infection [2]. Recent studies have highlighted the richness of essential oils, including essential oil of pistachio lentisque (EOP) (*Pistacia lentiscus* L., Anacardiaceae) containing various compounds known for their antibacterial and antioxidant properties [3]. In this context, the aim of our work was to design a fully biobased wound dressing containing EOP for use against pressure ulcers. On one hand, flax (*Linum usitatissimum* L., Linaceae) was chosen for two of its components: the flax fibers were processed for weaving a bio-functional support, and the flax seed hydrogel (FSH) was extracted and used as a viscous aqueous base. On the other hand, pistachio lentisque was used for the EO extracted from its leaves. The antioxidant activity of EOP (DPPH assay) was shown to be similar to that of 1g/l ascorbic acid, whereas the antibacterial activity of EOP (disk diffusion test) led to growth inhibition of *Staphylococcus aureus* and *Bacillus cereus*. The EOP was dispersed in the FSH to provide EOP/FSH emulsions at various amounts (0.1 % up to 5 %) using Ultraturrax®. All the surfactant-free emulsions had droplet sizes of less than 20 µm and stayed stable up to 45 days. The EO/FSH emulsions were spread on the woven flax fiber-based support and exhibited good adhesion properties on both the support and the skin. Hence, these findings underlined the potential of this fully biobased dressing against pressure ulcers (► Fig. 1).



► **Fig. 1** Exploded view drawing of the biobased wound dressing: (a) Essential oil (EO) droplets from pistachio lentisque; (b) Flax seed hydrogel (FSH); (c) Woven flax fibers. EO droplets not to scale.

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P387 Evaluation of essential oils (Ginger, Cinnamon, Thyme, Lavender, Arborvitae, Oregano, Tea-Tree, Marjoram, Sandalwood) in combination vincristine or doxorubicin in leukemic cell lines

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The use of essential oils as complementary therapies in oncology has gained interest due to their potential synergistic effects with conventional chemotherapy, although their specific efficacy against acute lymphoblastic leukemia (ALL) remains poorly characterized [1]. This study focuses on identifying potential complementary effects essential oils, aiming to enhance treatment efficacy of doxorubicin or vincristine, while potentially reducing toxicity. We evaluated the effects of several essential oils including Ginger (*Zingiber officinale*), Cinnamon (*Cinnamomum zeylanicum*), Thyme (*Thymus vulgaris*), Lavender (*Lavandula angustifolia*), Arborvitae (*Thuja plicata*), Oregano (*Origanum vulgare*), Tea tree (*Melaleuca alternifolia*), and Marjoram (*Origanum majorana*) on RS4;11 and SUP-B15 ALL cell lines. Cell viability was evaluated by Annexin V/Zombie Aqua staining and flow cytometry, while metabolic activity was measured using WST-1 assays, to determine the half-maximal inhibitory concentrations (IC₅₀). Selected oils were then combined with vincristine (11 µg/mL) or doxorubicin (1 µM), concentrations previously established to achieve IC₅₀. Notably, combinations of vincristine with tea tree, marjoram, or ginger exhibited enhanced cytotoxicity in both cell lines. Similarly, doxorubicin combined with oregano, thyme, ginger, or cinnamon demonstrated significant additive effects. Additionally, oregano, cinnamon, ginger, and sandalwood displayed high cytotoxicity as single agents. These findings suggest that certain essential oils may hold therapeutic potential as complementary agents in ALL treatment. Further studies are needed to evaluate their selectivity and potential cross-toxicity. This preliminary research opens new avenues for developing more effective and potentially less toxic combination therapies for leukemia.

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P388 Protective effect of two essential oil compounds, linalool and geraniol, on neurons in *In Vitro* Parkinson's Models

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Parkinson's disease is one of the most prevalent neurological disorders affecting millions of people worldwide [1]. There is a growing demand for novel and natural substances as complementary therapies. Essential oils and their various compounds are highly investigated natural plant-based products as potential treatment options for common human diseases, such as microbial infections, chronic diseases, and neurodegenerative disorders [2]. Several molecular mechanisms, including inflammation and oxidative stress may contribute to the etiology and progression of neurodegenerative diseases [3]. The present study focuses on the beneficial effects of linalool and geraniol, the major compounds of lavender (*Lavandula angustifolia* L.) and geranium (*Pelargonium graveolens* L'Hér. in Aiton) essential oils, on oxidative stress, inflammation, and iron metabolism of the rotenone and 6-hydroxydopamine-induced *in vitro* Parkinson's models. The experiments were carried out on alltrans retinoic acid differentiated SH-SY5Y cells. The effects of linalool and geraniol were compared to rasagiline, a monoamine oxidase B inhibitor. The results revealed that both essential oil compounds reduce the level of reactive oxygen species and alter the antioxidant capacity of the cells. They lower the secretion of IL-6, IL-8, and IL-1β pro inflammatory cytokines. Moreover, linalool and geraniol change the expression of iron-related genes, such as the iron importer transferrin receptor 1, heme-oxygenase-1, and ferroportin iron exporter, and influence the intracellular iron contents. In addition, it has been unveiled that iron availability is concatenated with the actions of the essential oil compounds. Based on the results, linalool and geraniol are vigorous candidates as an alternative therapy for Parkinson's disease.

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P389 Evaluation of the wound healing potential of volatile oil-loaded nanofibers

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Volatile oils possess antimicrobial and anti-inflammatory activities beneficial for wound healing. In time, nanotechnological approaches like nanofibers have been developed to enhance volatile oils' stability, bioavailability and controlled

release [1] Our study aims to develop nanofibers loaded with a mixture of volatile oils to utilize in wound healing.

A volatile oil mixture was prepared according to the book of Demirezer et al. [2]. Four volatile oils were used namely; pelargonium, chamomile, immortelle and tea tree oils were used as volatile oils to prepare the mixture. Polyvinylpyrrolidone (PVP) 360 was used for volatile oil-loaded nanofiber production. Formulations with polymer concentration of 15 % (w/v) and 1 ml of volatile oil mixture were electrospun. The experimental setup varied in voltage, flow rate and collector distance. GC-MS analyzes of the mixture of oil and nanofibers were conducted. While the microbroth dilution method was applied to both volatile oil and the mixture oil, disc diffusion method used for the nanofiber parts. Strains were *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* ATCC 10231 [3, 4]. The results of antimicrobial tests are given in Table 1. The nanofibers collected in the collector formed a circle shape with a diameter of 5 cm in the production where a homogeneous fiber throw occurred. Only 4-terpinol, neryl acetate, geraniol and citronellol are found in GC-MS results of nanofibers. When all results were evaluated jointly, mixture volatile oil loaded nanofibers can be promising for wound healing activity however additional experiments should be designed by changing the parameters.

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P390 Chilean *Aloysia* essential oils: A medicinal plant resource for postharvest disease control

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Plant pathogenic fungi, such as *Botrytis cinerea* and *Monilinia spp.*, cause substantial pre- and post-harvest losses, raising food safety concerns due to mycotoxin production [1]. Current control methods using synthetic fungicides face increasing resistance and environmental risks. This necessitates exploring sustainable alternatives like essential oils from medicinal plants for safer and more effective disease management. Essential oils from medicinal and aromatic plants are emerging as a promising alternative due to their lower toxicity and biodegradability. This research focuses on *Aloysia citrodora* (Lemon Verbena) and *Aloysia polystachya* (Burrito), South American aromatic plants with antifungal and antibacterial potential [2, 3]. However, there is a lack of information regarding the chemical composition and biological activities of their essential oils in combating postharvest fungi, especially in specimens cultivated in the central arid zone of Chile, justifying the need for further investigation. In this study, the chemical composition and efficacy of essential oils extracted from *A. citrodora* and *A. polystachya* and their respective major compounds against postharvest rot fungi in stone fruits were investigated. The GC-MS analysis of essential oils revealed compositional differences. *A. citrodora* is characterized by spathulenol and caryophyllene oxide as major components, along with smaller amounts of geraniol and farnesol. Conversely, *A. polystachya* is distinguished by the dominance of carvone, followed by dihydrocarvone and li-

monene. The antifungal activity of the essential oils and their major compounds was evaluated, highlighting the effectiveness of *A. citrodora* oil and farnesol against *M. fructicola* and *B. cinerea*. Against *M. fructicola*, *A. citrodora* oil and farnesol exhibited EC50 values of 61.89 µg/mL and 72.18 µg/mL, respectively, with conidial inhibition exceeding 75 %. Against *B. cinerea*, the EC50 values were 85.34 µg/mL (*A. citrodora* oil) and 47.6 µg/mL (farnesol), with conidial inhibition surpassing 50 %. These results suggest that *A. citrodora* oil holds significant potential for application in the agricultural industry. Given its comparable effectiveness to natural fungicides like BC1000, its action could be further enhanced by loading it onto nanoemulsions and combining it with farnesol to potentiate its activity.

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P391 Antifungal activity and composition of the essential oil from leaves of female and male trees from *Pimenta dioica* L. Merrill

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The fruits of allspice (*Pimenta dioica* L. Merrill, Myrtaceae) possess mixed aromatic notes, for instance, nutmeg, clove, black pepper, and cinnamon. This species is dioecious, thus, only female trees are the only fruit-bearing specimens, while male trees function only for pollination. The sexual differentiation between them is notorious only after 5 years of growth, when the first flowering and fruiting occur [1]. Then, male trees are removed from the field, and their leaves are used as only as cooking condiment. Nonetheless, male leaves also possess essential oil vesicles, which provide them with potential as a source of essential oils [2]. Those essential oils might also have agronomic applications. Therefore, the aim of this study was to determine and compare the essential oil composition and the antifungal activity against *Fusarium oxysporum* between essential oils obtained from leaves of female and male trees. The volatile components were analysed by GC-MS. For antifungal tests, 250 mg/L was employed for all essential oils. The GC-MS profile indicated specific volatiles to males and females, for instance, isocumene exclusively found in males, which has been reported with fungistatic activity. This was confirmed by antifungal in vitro tests, where the fungal growth was mainly reduced but not totally stopped in most cases. Interestingly, male essential oils were more active than female essential oils. Thus, non-used male trees represents a valuable source of bioactive essential oils with potential antifungal application.

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P392 Composition and biological activity of bergamot essential oil

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Inflammatory bowel disease (IBD), encompassing conditions such as Crohn’s disease and ulcerative colitis, presents significant public health concern. Recent scientific interest has focused on the pharmacological properties of essential oils, particularly bergamot (*Citrus bergamia* Risso et Poiteau) essential oil (BEO) [1]. This study aimed to investigate the biocompatibility limits, through ecotoxicological models (allelopathy assay, *Artemia salina* lethality, *Daphnia magna* cardiotoxicity assay), and protective effects in C2C12 cells and in isolated mouse colon specimens, of a commercial bergamot essential oil characterized for the content in linalool, linalyl acetate, and limonene. In the allelopathic assay, high BEO concentrations (125–1000 µg/mL) significantly inhibited germination and growth of *Chichorium intybus*; thus, indicating phytotoxicity. Conversely, lower concentrations (under 100 µg/mL) improved germination and growth, indicating minimal phytotoxicity and good biocompatibility. The *A. salina* lethality assay classified BEO as toxic to highly toxic with LC50 value > 5.67 µg/mL. By contrast, in the *D. magna* the same concentration did not modify heart rate beat; thus, scaling back the toxicity. This observation was also confirmed by the null effect of BEO (1–100 µg/mL) on C2C12 cell viability, and the anti-inflammatory effects isolated mouse colon tissue exposed to LPS. In this ex vivo model, BEO treatment (1–100 µg/mL) increased the expression of the anti-inflammatory cytokine IL-10 while reducing pro-inflammatory cytokines IL-6 and TNF-α. Overall, the present findings suggest that bergamot essential oil holds promise as a therapeutic option for managing inflammatory bowel disease. Its anti-inflammatory, antioxidant, and protective properties warrant further investigation to elucidate the underlying molecular mechanisms.

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P393 Antibacterial activity of fennel (*Foeniculum vulgare* Mill.) essential oil against uropathogenic *Escherichia coli*

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Nosocomial uropathogens represent a growing clinical concern due to their high resistance to antibiotics and their role in complicated, hospital-acquired urinary tract infections [1]. Therefore the elimination of bacterial biofilms continues to pose a significant challenge in healthcare system [2]. The aim of this study was to investigate if fennel (*Foeniculum vulgare* Mill.) essential oil (FEO)

could eradicate the biofilm formed by *Escherichia coli*. The other purpose was to investigate if fennel essential oil has efflux-pump inhibition ability [3]. To determine the exact composition of FEO, analytical test (GC-MS) was performed. The main component of FEO was trans-anethole (73.1 %). Antibiotic sensitivity of test bacteria was also investigated. Minimum inhibitory concentration (MIC = 0.31 mg/mL) was determined with microdilution method. Crystal violet assay was used to evaluate the antibiofilm effect of FEO, and inhibition rate value was calculated (84.3 %) [4]. To investigate the efflux-pump inhibition ability, fluorescence activity was monitored at excitation and emission wavelengths of 525 and 615 nm every minute for one hour on a real-time basis. Relative fluorescence index (RFI) was calculated (FEO = 1.09) and compared to the positive control (CCCP = 0.91). Our *in vitro* results showed antibiofilm activity and efflux-pump inhibition ability of FEO, and highlighted that the use of FEO should be considered to prevent the spread of uropathogens. After further *in vivo* examinations it might be supposed to use FEO against nosocomial pathogens in clinical practice.

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P394 Anticancer potential of Cinnamon essential oil against HPV-Positive and HPV-Negative cervical cancer cells: differential antiproliferative, proapoptotic, and senescence-inducing effects

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Cinnamon essential oil (CEO) has demonstrated significant bioactive properties, including anticancer potential [1–5]. These properties make it a promising candidate for therapeutic applications. It is worth noting that its effect on cervical cancer has been poorly explored. Therefore, this study evaluates the impact of CEO on HPV-positive (HeLa, SiHa) and HPV-negative (C33A) cervical cancer cell lines compared to non-tumorigenic cells (HaCaT). Specifically, this study aims to assess the effects of CEO on cell viability, proliferation, apoptosis induction, mitochondrial dysfunction, and senescence in cervical cancer versus non-tumorigenic cells. Cell viability was measured using the WST-1 and sulforhodamine B assays. Apoptosis was evaluated by Annexin V/Propidium iodide staining, while caspase-3, -8, and -9 activation was determined through fluorescein isothiocyanate (FITC) staining. Mitochondrial membrane potential (ΔψM) was analyzed using JC-10 staining. Proliferation was assessed by 5-Ethynyl-2'-deoxyuridine (EdU) incorporation, cell morphology was examined microscopically, and senescence was evaluated using senescence-associated β-galactosidase (SA-β-gal) staining. CEO exhibited higher toxicity in cervical cancer cells than in HaCaT cells, with HeLa being the most sensitive (IC₅₀ = 1:40,000). CEO induced dose-dependent apoptosis, confirmed by

caspase activation and mitochondrial depolarization. A strong antiproliferative effect was observed, particularly in HeLa and C33A cells. Senescence was significantly induced in HeLa after 72 hours and in C33A after 48 hours. In summary, CEO selectively affects cervical cancer cells by reducing cell viability, inducing apoptosis, promoting cellular dysfunction, and inducing senescence, with minimal effects on non-tumorigenic cells. These findings suggest CEO as a potential complementary therapeutic agent for cervical cancer treatment.

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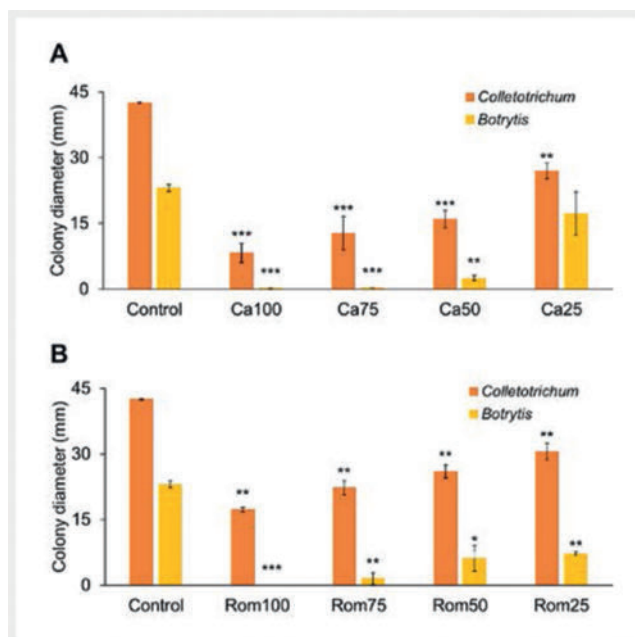
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P395 The *in vitro* potential of essential oils for controlling postharvest fungi

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Fungal diseases are among the most significant factors affecting food quality during transportation and storage, particularly in the case of fresh fruit intended for export over long distances. These diseases are commonly managed with chemical fungicides, which can be toxic to human health and contribute to the development of fungicide resistance. In this context, alternative and less harmful management strategies must be developed [1]. Therefore, the aim of this research was to characterize the chemical composition and antifungal activity of common essential oils against postharvest fungi. The oils were extracted by (► Fig. 1) hydrodistillation and tested *in vitro* against *Botrytis* sp. and *Colletotrichum* sp. Rosemary and cinnamon essential oils were the most effective against both pathogens. GC-MS analysis revealed the presence of several mono- and sesquiterpenoids; however, the oil composition was dominated by two or three major components. *In silico* analyses [2] of the main volatile compounds indicated a variety of potential mechanisms of action against fungi, such as cell membrane disruption, sugar-phosphatase inhibition, and fatty-acyl-CoA synthase inhibition, among others. Therefore, essential oils show great potential for the management of postharvest diseases, and *in silico* analysis could serve as a useful tool for guiding the selection of oils for this purpose.



► **Fig. 1** Fungal colony size without exposure to essential oils (control) and exposed to rosemary (Ro) and cinnamon (Ca) in four concentrations 100: 1000 mg/L; 750: 750 mg/L; 50: 500 mg/L; 25: 250 mg/L at the fifth day of fungal growth.

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P396 Eco-friendly weed control: herbicidal potential of *Achillea millefolium* essential oil

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Extensive reliance on synthetic herbicides became a significant concern, with implications for human health and the environment. Furthermore, the increasing development of resistance among weed species has reduced the effectiveness of traditional herbicide treatments. Consequently, plant-derived compounds are increasingly being explored as natural alternatives for pest and weed management, mainly due to their selectivity, biodegradability into non-toxic products and minimal impact on non-target organisms and ecosystems [1, 2]. This study aimed to: i) analyze the phytochemical profile of *A. millefolium* essential oil (EO); ii) evaluate its potential phytotoxic effects on the seeds of *Sinapis arvensis* L., *Hordeum vulgare* L., *Raphanus sativus* L. and *Lolium multiflorum* Lam.; iii) extract and evaluate α -amylase activity from treated seedlings to investigate a possible correlation between phytotoxicity and anti-amylase activity; iv) evaluate ecocompatibility through *Artemia salina* assay; v) explore antimicrobial properties against some bacterial and fungal phytopathogenic strains. Chemical composition analysis conducted by GC-MS identified 69 compounds, with oxygenated monoterpenes as the dominant group, including

borneol (12%), camphor (11.5%) and 1,8-cineole (8.9%). EO demonstrated phytotoxic effects on seed germination and seedling growth, particularly against *S. arvensis*. Subsequent to the phytotoxic activity, a reduction in the α -amylase activity extracted from the seeds was observed, suggesting a possible correlation between the two activities. Furthermore, EO was not toxic on *Artemia salina*, suggesting its potential as a safe bioherbicide. EO also showed antimicrobial activity against *X. campestris* and *P. italicum*. These results highlight the potential of *A. millefolium* EO as a natural herbicide and antimicrobial agent, offering an environmentally friendly alternative for sustainable agriculture.

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P397 Insights into plant adaptation and environmental radioactivity in the Nirano natural reserve (AG): A multidisciplinary study

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Sedimentary volcanism is the surface manifestation of subterranean processes, including mud volcanoes which can pose geohazards during events such as gas blasts and mud ejections. The gas released is methane, with small amounts of CO₂, N₂ and light hydrocarbons. Salse di Nirano, a nature reserve of mud volcanoes in Emilia-Romagna (Italy), hosts a plant association that can survive in such conditions. This study, part of the PROMUD (<https://progetti.ingv.it/it/promud>) project, investigates the resilience of the halophyte *Atriplex sagittata* Borkh. collected from Ferdinando (the oldest volcano) and the young Egidio volcanoes. A multidisciplinary approach was used to study the interaction between seepage dynamics, fault activity and plant metabolism, with the aim of establishing a link between the geomorphological evolution of the cones and the metabolism of halophytic species. Firstly, a phytochemical characterisation of *A. sagittata* was performed using HRESIMS. This analysis led to the identification of 64 compounds, including flavonoids, phenolics derivatives, phenylethylamine alkaloids, and sulphated flavonoids [1]. Metabolomic analysis showed that *A. sagittata* from the Fernando and Egidio mud volcanoes had similar profiles, with the exception of sulphated flavonoids and alkaloids, which were found only in the Egidio population. Water samples from the mud volcanoes show significant differences in ionic composition, with higher levels of sodium, chloride and bromide at the more frequently flooded Egidio site, suggesting that salt input is key. These results highlight how *A. sagittata* can survive in extreme volcanic environments and how its specialised metabolites may play an important role in stress resistance research [2].

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P398 Comprehensive profiling of phytocannabinoids and phenolic compounds in hemp seeds, flours, and oil

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Hemp products such as seeds, flours, and oils are increasingly sought-after due to their favorable nutritional composition. In addition to a high content of unsaturated fatty acids in the lipid fraction, these products are a rich source of phenolic compounds [1]. Besides such secondary metabolites with antioxidant potential, the hemp plant contains several other unique molecules, including cannflavins possessing this bioactivity. Also, phytocannabinoids can be transferred from inflorescence to the surface of seeds/products thereof, thus contributing to their bioactivities, both beneficial (antioxidant, neuroprotective, antimicrobial) and adverse (psychotropic). In this study, the content of 47 phytocannabinoids was determined in samples of hemp seeds (31 samples; total phytocannabinoids: 1–81 mg/kg), flours (13 samples; 4–197 mg/kg), and oils (43 samples; 0–1260 mg/kg), using ultra-high-performance liquid chromatography coupled with tandem high-resolution mass spectrometry (UH-PLC-HRMS/MS). In 69 samples, the predominant phytocannabinoid was cannabidiol/cannabidiolic acid (CBD/CBDA); in 14 samples, it was Δ 9-tetrahydrocannabinol/ Δ 9-tetrahydrocannabinolic acid (Δ 9-THC/ Δ 9-THCA); and in 4 samples, it was cannabigerol/cannabigerolic acid (CBG/CBGA). Alongside the psychotropic *trans*- Δ 9-THC (detected in 95 % of samples), not the routinely detected *cis*- Δ 9-THC isomer (detected in 37 % of samples) was also quantified; in some cases, it accounted for up to 50 % of the total THC equivalent. In 8 samples, the legal limit for Δ 9-THC content, as set by Regulation (EU) 2023/915 on maximum levels for contaminants in food, was exceeded. In addition to phytocannabinoids, the profile of phenolic compounds was also assessed. The most abundant compounds included cannflavins A and B, apigenin, quercetin, catechin, salicylic acid, ferulic acid, p-coumaric acid, and caffeic acid.

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P399 One sample, two stories: A comparative dive into NMR and LC-HRMS metabolomics in natural products and foods

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Metabolomics has emerged as a powerful approach for characterizing the chemical complexity of natural products and foods, enabling the discovery of bioactive compounds, assessment of nutritional quality, and authentication of sources. Among the arsenal of analytical tools available, NMR spectroscopy and LC-HRMS are widely regarded for their complementary strengths. This study presents a comparative investigation of these two platforms using identical samples derived from diverse natural matrices, i.e. leaves from *Pistacia lentiscus* var. *Chia*, honey and table olives, showcasing how each technique offers a distinct narrative of the metabolome. NMR spectroscopy excels in delivering highly reproducible, quantitative data with minimal sample preparation and provides direct structural information. In contrast, LC-HRMS boasts superior

sensitivity, allowing the detection of a vast array of low-abundance and chemically diverse metabolites, albeit often with challenges in quantitative information and structural confirmation. By applying both methods to the same set of samples, key differences in metabolite coverage, compound annotation rates, and data complexity were highlighted in the different matrices [1–3]. Through non-targeted metabolite profiling and multivariate analysis, we demonstrated how NMR and LC-HRMS each uncover unique aspects of the metabolic landscape. This dual-platform approach underscores the importance of methodological complementarity in metabolomics, ultimately enhancing the metabolite annotation confidence. Through this “one sample, two stories” paradigm, we propose integrative workflows that exploit the strengths of both NMR and LC-HRMS to unravel the full complexity of metabolomes in natural and food products.

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P400 *Withania somnifera* (Ashwagandha): is reported toxicity linked to poor quality?

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There have been reports of liver damage linked to *Withania somnifera* (L.) Dunal. These reports have caused several countries to ban the use of this plant. Adverse liver reactions can be intrinsic or idiosyncratic and include possible adulteration. Known adulterants include aflatoxins, pyrrolizidine alkaloids, heavy metals and incorrect species or incorrect plant part. Moreover *W. somnifera* is traditionally produced as an aqueous extract and using different extraction techniques may alter the known safety profile. The aim of this investigation was to highlight quality deficiencies in these products. We sampled *W. somnifera* from internet sources and subjected the samples to a comprehensive quality analysis. Samples were analysed using HPTLC, NIR and HPLC methods, comparing strengths and weaknesses of the different techniques. We also analysed for tablet hardness and friability and checked samples for label compliance. The *W. somnifera* samples showed marked variation in both composition and concentration of metabolites. Some samples contained very low amounts of important withanolides. Over 90% of the samples did not conform to national requirements for labelling, including making illegal claims.

The results highlight poor quality and variability of products available through the internet. Although no leaf material was found in the root samples, our analysis showed that different extraction techniques resulted in a different chemical profile to the aqueous extraction profile that is traditionally used for this plant. The quality of *W. somnifera* products is highly variable and different extraction methods produce end products that contain different compounds to the traditionally produced KSM66 aqueous extract [1].

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P401 Essential oil chemical profiling as a tool for selecting pathogen-resistant Genovese-type basil with desirable aromatic traits

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Genovese-type basil (*Ocimum basilicum* L.) is a key ingredient in Mediterranean cuisine, particularly in the preparation of “pesto” sauce, due to its distinctive sweet and floral aroma [1]. The increasing demand for fresh basil leaves from the food industry has prompted Italian farmers to expand basil cultivation, with a total production exceeding 8,000 tons in 2024 [2]. However, its high susceptibility to fungal pathogens limits its cultivation and economic potential [3]. This study aimed to combine the peculiar aroma of Genovese-type basil with pathogen resistance through the chemical analysis of essential oils (EOs) from basil genotypes derived from the hybridization of resistant basil lineages with the classic Genovese one. Fresh basil leaves were subjected to hydrodistillation for EO extraction, followed by gas chromatography-mass spectrometry (GC-MS) analysis to determine their volatile composition. The main identified chemical classes were terpenes and phenylpropanoids, with linalool and eugenol as the dominant compounds. Three distinct clusters were identified: (1) genotypes with low eugenol and high oxygenated sesquiterpenes, (2) control Genovese-type basil with high linalool and *trans*- α -bergamotene, and (3) hybrid genotypes with increased eugenol and reduced sesquiterpene hydrocarbons. The hybrid genotypes with the most similar aroma features to the classic Genovese one were identified. These findings highlight the potential for chemical analysis to support basil breeding programs, enabling the selection of hybrid genotypes that balance disease resistance with desirable aromatic profiles. The study provides a foundation for further investigations into optimizing Genovese-type basil cultivars for industrial applications while maintaining their characteristic sensory attributes.

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P402 Chemical fingerprints of 10 *Capsicum* varieties through non-targeted UHPLC-HRMS analysis combined with a chemometric approach: a first step towards fraud detection and prevention

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Chili peppers belong to the Solanaceae family and the *Capsicum* genus [1]. It is one of the most widely consumed vegetables in the world, primarily due to its sensory attributes, as well as its rich content of nutrients such as minerals, vitamins, phenolic acids, and flavonoids [1]. Furthermore, Chili peppers have been found to be among the spices most subject to manipulation, substitutions, adulterations and/or contamination [2]. The aim of this research was to establish a link between varieties and chemical fingerprints of 10 *Capsicum*

varieties, through an untargeted metabolomics approach based on comprehensive UHPLC-Q-Orbitrap-HRMS fingerprinting combined with multivariate data analysis. The samples were classified based on classical taxonomy, considering morphological similarities such as: shape, color and dimension. UHPLC-HRMS fingerprinting combined with a chemometric approach, demonstrated strong predictive power, enabling clear differentiation of clusters within the investigated categories. The main compounds detected were: phenolic (glycosylated hydroxycinnamic acids), flavonoid glycosides (luteolin, quercetin, apigenin and naringenin derivatives), capsaicinoids and carotenoids. Capsaicinoids detected in the chili pepper samples were: capsaicin, dihydrocapsaicin, homodihydrocapsaicin and nordihydrocapsaicin. Capsaicin and dihydrocapsaicin were quantified by UHPLC-UV. The highest amount of capsaicinoids was detected in samples: Carolina Reaper, Moruga, Bhut Jolokia and Naga chocolate. These findings are in accordance with the Scoville Scale. The specific color a pepper exhibits depends on the combination of carotenoids present in it. Capsanthin, capsorubin and cryptocapsin were detected in red chili pepper, while they were loss in yellow chili pepper. In the latter, the predominant carotenoids were lutein, β/α carotene and zeaxanthin.

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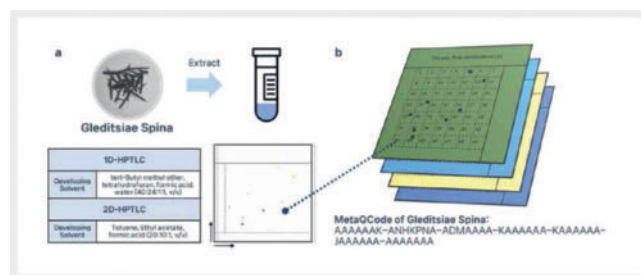
P403 A study for applicability of MetaQCode system, constructed by using 2D-HPTLC

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In the realm of modern herbal medicinal products, the quality control and standardization of medicinal plants have become paramount, driven by advancements in analytical methodologies. Thus, we present a pioneering approach to analyze and profile medicinal plants, called “MetaQCode”, using two-dimensional high-performance thin layer chromatography (2D-HPTLC) combined with a novel systematic coding framework. This method facilitates characteristic metabolomic profiling under standardized extraction and analysis conditions, offering a unique coding system for quality assurance. Our research focuses on assessing the applicability of this nascent system by applying it to medicinal plants belonging to the Leguminosae family such as *Gleditsiae Spina* (GS), *Gleditsiae Fructus* (GF), *Sophorae Fructus* (SFr), and *Sophorae Flos* (SF). Through 2D-HPTLC analysis, we extracted and visualized four distinct data sets per sample. Subsequently, we defined valid grids, identified relevant spots, and assigned scores, which were then transformed into alphabetic characters through our systematic coding process, culminating in the generation of a unique code termed “MetaQCode” for each sample. Furthermore, we evaluated the system’s effectiveness by measuring the similarity

between MetaQCodes of GS and GF, as well as between SFr and SF, using Euclidean distance. Our results confirmed that the MetaQCode system effectively differentiated between GS and GF, as well as between SFr and SF (► Fig. 1).



► **Fig. 1** An example of *Gleditsiae Spina* extraction and 2D-HPTLC analysis with a standardized condition (a), and assignment of MetaQCode to *Gleditsiae Spina* (b).

Despite the challenges inherent to its developmental stage, this study underscores the potential of this system. With further refinement and systematization, this innovative approach holds promise for advancing metabolome-based classification technologies and enhancing the quality control mechanisms for medicinal plants.

P404 Predicting *Camellia* genus flower traits using leaf metabolomics and machine learning approaches

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Artificial intelligence (AI), and in particular machine learning (ML), is revolutionising plant science by enabling predictive models to be created from complex omic datasets, notably metabolomics. For slow-growing species, such as *Camellia*, an emblematic genus in cosmetics, horticulture, and food industry, ML offers a unique opportunity to accelerate varietal selection and screening for bioactive potential without waiting for phenotypic maturity [1]. This study explores the predictive potential of leaf metabolomics for anticipating floral characteristics and biological activities of interest. More than 2,000 samples, representing more than 300 genotypes and around twenty species of the *Camellia* genus, were analysed using reverse-phase liquid chromatography coupled with Orbitrap high-resolution mass spectrometry (LC-MS, negative ion mode). The metabolomic profiles obtained from the leaves were used to train models capable of predicting flower shape and colour with an accuracy of over 80 %, this model could be applied to young plants by predicting phenotype traits up to 3-5 years before flowering. Predictive models were validated using different independent sampling years. The metabolic predictor identified include more than a dozen chemical families, dominated by phenolic compounds, lipids, amino acid derivatives, and benzoin-type compounds. This approach and the richness of the dataset acquired can also be used to predict new parameters related to the plant, its crop or the biological activities that could be associated with it.

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P405 Development and validation of an LC-MS analytical method for the quantification of cannabinoids in blood plasma of medicinal CBD users

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The *Cannabis sativa* L. plant has been used worldwide for medicinal purposes for thousands of years and is a rich source of bioactive compounds, such as cannabinoids. Among them, Δ^9 -THC and CBD stand out for their therapeutic effects, with applications in pain relief, and the treatment of several health conditions. CBD, in particular, has shown potential in the treatment of knee osteoarthritis, relieving pain and improving patient mobility [1]. Thus, this study aims to establish an analytical method for the quantification of CBD, CBN, Δ^9 -THC, CBG, 11-OH-THC and 11-COOH-THC in 40 blood plasma samples, contributing to better dosage control and monitoring of cannabinoid therapy. To evaluate the selectivity of the method, a matrix effect study was conducted, in which a t-test indicated that the slopes of the calibration curve did not present statistically significant differences for the cannabinoids analyzed. The calibration curves covered a concentration range of 0.0025 to 0.25 $\mu\text{g mL}^{-1}$, demonstrating good linearity, with correlation coefficients (R^2) ≥ 0.9855 . The limits of quantification (LOQ) ranged from 4.10×10^{-2} to $2.14 \times 10^{-2} \mu\text{g mL}^{-1}$, while the limits of detection (LOD) were between 1.19×10^{-2} and $9.42 \times 10^{-3} \mu\text{g mL}^{-1}$. The results indicate that the developed method presents good selectivity and linearity, together with LOD and LOQ values appropriate for biological matrix analysis. The absence of matrix effect reinforces its reliability. However, the validation process is still ongoing, with precision and accuracy assessments, critical steps to ensure the robustness and applicability of the method in routine analytical use.

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P406 ^{13}C Enriched duckweed as a platform for structural elucidation of plant metabolites

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The *Lemnaceae* family comprises 36 species of simple aquatic plants commonly known as duckweed. Interest in *Lemnaceae* has returned due to the high-quality genome mapping of *Spirodela polyrrhiza* (*S. polyrrhiza*), and 9 additional duckweed species, affording new opportunities for complementary experimental approaches aimed at better characterizing this rising model organism [1] [2]. Notably, duckweed's amenability to grow under auxotrophic conditions makes it uniquely suited for developing methodologies for natural product research and complex structural elucidation of plant metabolites. Cultivating *S. polyrrhiza* in the dark on Schenk and Hildebrandt liquid media supplemented with 0.5 % ^{13}C -glucose yielded 96 % ^{13}C enrichment in the cell wall, and widespread iso-

topic labeling of metabolites. The ^{13}C enriched biomass was extracted, and a baseline suite of highly resolved one- (1D) and two-dimensional (2D) nuclear magnetic resonance (NMR) spectra were collected. High-performance liquid chromatography (HPLC) separation of the duckweed extract enabled the generation of a 1D ^{13}C NMR “fraction library”. Complex fractions were computationally selected for further analysis to maximize structural elucidation of carbohydrates by collecting advanced 2D NMR experiments which included INADEQUATE (incredible natural abundance double-quantum transfer experiment) and HETCOR (heteronuclear correlation). This methodology demonstrates an emerging technique by which duckweed can be used to define plant metabolite networks at the systems level. With a comprehensive metabolite reference data library, *S. polyrrhiza* will serve as a powerful platform that can be leveraged in future studies encompassing natural product discovery, genetic engineering, biofuels, and other studies that rely on understanding the metabolite architecture of this humble aquatic monocot [2].

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P407 Dried spot microsampling in metabolite profiling: A novel approach for authenticating red and white wines

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The characterization of bioactive constituents in plant-derived foods requires advanced analytical methods to ensure quality and consumer safety [1]. Being a rich source of bioactive compounds, wine is a complex matrix and a highly commercialized product requiring efficient quality monitoring [2]. However, conventional approaches typically include laborious and resource-intensive sampling and sample preparation steps. On the other hand, dried blood spots (DBS) constitute a well-established microsampling technique where a small sample volume is spotted onto paper cards and dried before analysis, offering significant advantages including minute sample size, convenient sampling, and easy sample transportation via post [3]. In that context, this work integrates for the first time dried spot sampling in characterization of food commodities, combined with untargeted LC-HRMS/MS. Red as well as white wine were used as proof-of-concept. An optimized workflow for chemical profiling of wine was developed, involving volumetric microsampling of wine on DBS cards, followed by automated spot extraction and analysis. The proposed methodology was successfully compared to conventional sample preparation techniques for wine, providing similar chemical profiles. Several parameters were optimized for the extraction of the wine dried spots to achieve enhanced analytical sensitivity and wide metabolite coverage. Overall, the developed methodology may serve as an attractive solution for resource-efficient wine quality and authenticity control, featuring automated dried spot extraction with the possibility of on-site sampling by non-trained users, and is envisioned to pave the way for more accessible food quality assessment platforms.

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P408 Systematic fractionation using Complementary Developing Solvent technique in flash chromatography: A key step in dereplication strategies for natural products

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The Complementary Developing Solvent (CDS) technique, originally developed for High-Performance Thin Layer Chromatography (HPTLC), employs three solvent systems of varying polarities (low-polarity developing solvent, LPDS; medium-polarity developing solvent, MPDS; high-polarity developing solvent, HPDS) to achieve enhanced chromatographic resolution while covering a wide range of polarities and selectivities. This study demonstrates the successful adaptation of the CDS approach to normal-phase Flash chromatography, aiming to fractionate poorly studied plant extracts in a systematic manner and incorporate this approach into a broader dereplication strategy.

Through extensive optimization, we validated the feasibility of this adaptation for two out of the three solvent systems: LPDS (toluene/ethyl-acetate 100:0-85:15) and HPDS (dichloromethane/methanol 100:0-40:60), both performed as step-gradients. The same System Suitability Test (SST) employed in HPTLC – namely the Universal HPTLC Mix (UHM), a mixture of eight compounds covering a wide range of polarity – was used to enable direct comparisons between the two techniques. The elution profiles observed in Flash chromatography were consistent with those obtained in HPTLC. Compound identities and elution order were verified by collecting each chromatographic peak and manually matching MS² spectra against reference standards.

Additionally, we propose a systematic fractionation strategy of natural products by segmenting each elution gradient (LPDS, HPDS) into seven polarity “plateaus”, defined as distinct ranges of solvent polarity within the gradient where compounds with similar characteristics are likely to elute. This systematic approach of collecting a single fraction per plateau significantly reduces the number of samples for subsequent analysis, thereby accelerating the dereplication workflow.

P409 Highly variable proanthocyanidin contents in herbal medicinal products containing *Ginkgo biloba* leaf extracts from the European market

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In the last years a new analytical method for quantification of proanthocyanidins (PACs) in *Ginkgo biloba* leaf extract by HPLC has been published [1]. This method has recently been applied to the quantification of PACs in finished herbal medicinal products containing *Ginkgo biloba* leaf extract from the German market [2]. As significant differences were found, a new study was initiated that extended the analytical comparison of PAC content to herbal medicinal products containing *Ginkgo biloba* leaf extract from additional European countries. The results obtained so far indicate up to 10-fold differences in PAC content between finished medicinal products available in European countries with a range of approximately 0.5 % to 5 % in the *Ginkgo biloba* leaf extract. The highest contents were found in herbal medicinal products containing the *Ginkgo* extract EGb 761. This is relevant, as interest in PACs is currently increasing due to a variety of reported pharmacological activities. PACs are coming more and more into focus as a quality parameter for herbal medicinal products containing *Ginkgo biloba* leaf extract.

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P410 Uncovering adulteration in *Tribulus terrestris* supplements using LC-MS-based metabolomics

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Tribulus terrestris dietary supplements are widely consumed for their purported health benefits, yet concerns persist regarding product authenticity and the potential for adulteration. In this study, an untargeted liquid chromatography-high-resolution mass spectrometry (LC-HRMS) metabolomics approach was employed to investigate the composition of *T. terrestris*-derived supplements. The analysis encompassed authentic plant materials, intentionally adulterated samples, and commercially available products. Data interpretation using principal component analysis (PCA), orthogonal partial least squares discriminant analysis (OPLS-DA), and a convolutional neural network tool enabled the differentiation of sample groups and identification of irregularities. The presence of pharmacologically active adulterants, including PDE5 inhibitors and anabolic steroids, was confirmed in spiked samples. Furthermore, certain commercial products were found to contain undeclared compounds derived from green tea and citrus, presumably added to enhance stimulant effects and reinforce testosterone-related claims. Spirost-4-ene-3,12-dione was detected as a potential marker of steroidal saponin degradation, likely linked to improper storage or processing. These findings underscore the effectiveness of LC-HRMS-based metabolomics in detecting supplement adulteration and highlight the pressing need for stringent quality control measures to ensure consumer safety and product authenticity [1].

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P411 Optimization of a miniaturized and green ultrasound-assisted sample preparation method for HPLC quantification of phenolic acid and flavonoids in honey

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Honey is one of the most appreciated and valued natural products; it is used not only as a nutritional product but also in traditional medicine for the treatment of several clinical conditions [1]. Polyphenols are linked with diverse health effects and considered as markers of botanical and geographical origin [2,3]. The extraction of polyphenols from honey requires complicated and time-con-

suming extraction techniques due their low contents and matrix particularity [4]. The objective of this study was to develop and optimize the direct ultrasound-assisted solubilization of phenolic acids and flavonoids in honey samples before chromatographic separations. Thus, Box-Behnken designs have been conducted to study the effect of: (i) percentage of alcohol, (ii) temperature, (iii) solvent-honey ratio and (iv) sonication time on the contents of phenolic acids and flavonoids. The desirability index demonstrated that the maximum solubilization of honey flavonoids in pure methanol can be achieved by ultrasound of honey at 40 °C for 5 min using methanol at solvent-sample ratio of 10 mL x g. Regarding to phenolic acids, the use of 60.0% (v/v) ethanol at a solvent-honey ratio of 10 mL x g⁻¹ for a period of 17.8 min at a temperature of 54.6 °C resulted in their maximum solubilization. Furthermore, the optimized sample preparation methods were applied successfully for the analysis of multifloral honey produced in different districts of Cyprus island. In conclusion, the present work demonstrates novel, miniaturized sample preparation methods for the direct ultrasound-assisted solubilization of honey phenolic compounds at short time, using low volume of alcohols and small size of sample in comparison with existing methods.

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P412 Bee-yond Borders: building a comprehensive honey databank with chemometric insights

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Honey is a widely consumed natural sweetener, valued for its economic, nutritional and health benefits. The beneficial properties, however, vary significantly among different types of honey, making the assessment of its origin and authenticity crucial [1]. To address this, various analytical techniques have been developed to link honey's composition to its origin, revealing its unique traits. While several large-scale studies have focused on the sugar composition and volatile compounds [2], research on non-sugar metabolites remains relatively limited. The primary aim of this ongoing study is to analyze honeys from multiple countries, evaluate their composition, and establish a comprehensive global honey databank. So far, the workflow involves the extraction of over 300 samples using annular centrifugal extraction (ACE) followed by NMR metabolomics and multivariate analysis (MVA). A key advantage of NMR lies in its high reproducibility and its ability to analyze samples at different time points without being affected by batch effects [3] – an essential feature when sample collection can be time-consuming. Therefore, alongside the creation of a physical honey bank, a spectral data base is also being created based on NMR data. Additionally, in combination with MVA, a chemical mapping of honey is being generated. Statistical models reveal clusters of honeys with similar chemical profiles, demonstrating high robustness. Interestingly, even honeys harvested

from distant countries share similarities in their chemical composition. This project aims to include as many samples as possible from around the world, uncovering new honeys with unique compositions.

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P413 Development of microplate sealing lid SLIM-V for *in vitro* bioactivity testing of volatiles

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Volatile plant-derived products are a large group of carbon-based chemicals with low molecular weight and high vapor pressure at ambient temperature. They can be obtained from different plant parts in the form of essential oils, extracts, oleoresins, tinctures, distillates, and juice concentrates [1]. They were observed to exhibit a broad spectrum of biological effects including antimicrobial, anticarcinogenic, antioxidant, and cytotoxic properties [2]. However, due to their volatility, results of conventional microplate-based bioassays can be significantly affected by the vapors [3]. Recently, we designed a new sealing lid SLIM-V for microtiter plates to get reliable results of *in vitro* bioactivity screening of plant volatiles. In this study, the antibacterial activity of thymoquinone against *Staphylococcus aureus* was evaluated by using broth microdilution volatilization assay [4] and by its modification combined with SLIM-V with aim to validate its appropriateness. The results showed that the growth-inhibitory effect of thymoquinone in liquid and vapor phase was the same for both methods with the value of minimum inhibitory concentration 16 µg/mL in liquid and vapor phase. In summary, the presented sealing lid SLIM-V is suitable for susceptibility testing of bacterial pathogens to plant-derived volatiles. It can potentially be used in research of new pharmacological, medicinal, agricultural, and food preparations development based on volatile antimicrobials such as inhalation therapy, disinfection and sterilization gases in healthcare facilities, fumigants for controlled-atmosphere storage of agricultural products, and preservation agents for active or smart packaging of food products.

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P414 Effects of grain stabilization processing on the flavor and palatability of soybeans for functional food applications

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Soybeans are rich in bioactive compounds such as isoflavones, saponins, and phytosterols, which are known to help manage menopause, reduce cholesterol levels, and prevent hormone-related cancers. However, their broader application in functional and medicinal products is often limited by off-flavors, particularly the characteristic beany odor.

This study examined the effects of Grain Stabilization Processing (GSP) on volatile compounds and sensory attributes in both raw and roasted soybeans, using gas chromatography-mass spectrometry (GC-MS), electronic nose and tongue systems, and sensory evaluation.

GSP treatment increased the levels of alcoholic compounds, likely due to non-enzymatic oxidation of unsaturated fatty acids. In treated raw soybeans (TSB), heterocyclic compounds such as pyrazines and maltol were elevated, contributing to nutty and roasted aromas. In contrast, these compounds were decreased in treated roasted soybeans (TRSB), possibly due to thermal degradation. Electronic tongue analysis revealed significantly lower sourness, bitterness, and sweetness and higher umami intensity in TRSB compared to the control. Sensory evaluation indicated that TSB received significantly higher scores than raw soy bean (RSB) in terms of aroma, taste, mouthfeel, and overall acceptability. Correlation analysis revealed strong positive associations between overall preference and attributes such as roasted aroma, sweetness and roasted flavor, while negative correlations were observed for beany odor, bitterness and astringency [1, 2].

These findings suggest that GSP effectively improves the sensory quality of soybeans by reducing off-flavor compounds and enhancing desirable taste attributes. This approach may expand the potential use of soybeans in functional foods and medicinal formulations aimed at promoting health and preventing disease.

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P415 Non-targeted metabolomic approach for authenticity differentiation of shallots and onions using UHPLC-HRMS/MS and chemometrics

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Shallots (*Allium cepa* var. *aggregatum*) and onions (*Allium cepa* var. *cepa*) are subspecies of the *Allium cepa* that are marketed at different prices, the first being more valued for its more subtle flavor. However, authentication based on their morphological characteristics is often unreliable. The presented project aimed to design an analytical method applicable for distinguishing these two crops under routine inspection practice. Three sets of authentic shallots and onions, representing various cultivars, harvests (2022 and 2023), and storage conditions (short and long), were analysed by non-targeted metabolomic screening to identify authenticity markers and build classification models. Sample preparation and extraction were optimized for reverse phase ultra-per-

formance liquid chromatography coupled with high-resolution tandem mass spectrometry (UHPLC-HRMS/MS). Advanced chemometrics were applied on the obtained metabolomic data, using Orthogonal Partial Least Squares discriminant analysis (OPLS-DA) to discriminate the two groups, with the models achieving excellent predictive values. The Variance in Projection (VIP) score was used to select statistically significant markers, which were tentatively identified and assessed for their stability during storage and variation across different harvests. The proposed identifications included derivatives of sugars and amino acids, oligopeptides, flavonoids, steroids, and a derivative of the organosulfur compound alliin, which is typical of the *Allium* species. The final classification model, built using the selected markers, achieved a 100 % predictive performance upon external validation. Sugars and amino acids were determined to provide additional discrimination between shallots and onions, with sugars yielding a statistically significant screening value.

P416 Applying heterocovariance approach to discover antileishmanial compounds from *Artemisia umbelliformis* subsp. *eriantha*

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Metabolomics approaches have a major contribution in the study of natural products as they offer a combination of spectroscopic techniques and multivariate statistical methods, that significantly accelerate the analysis of a complex plant extract. In this study, recently developed Heterocovariance approach (HetCA) [1] along with Statistical Total Correlation Spectroscopy (STOCSY) are applied on *Artemisia umbelliformis* subsp. *eriantha* extract, in order to facilitate the detection of antileishmanial compounds. The extract was fractionated by Centrifugal Partition Chromatography (CPC), creating fractions that reflected fluctuation in the concentration of the different components. The 1H-NMR data of the fractions were recorded, as well as their bioactivity results against *Leishmania infantum* promastigotes. Spectral data were then statistically correlated with antileishmanial activity results by HetCA. HetCA along with STOCSY indicated sets of NMR signals correlated to the activity, that corresponded to specific compounds. Additionally, HetCA indicated sets of NMR peaks that showed non-correlation with the bioactivity. Both correlated and non-correlated compounds were isolated purposefully and were tested against *L. infantum* promastigotes, showing that the positively correlated compounds 5-deoxy-5-hydroperoxy-telekin (1) and telekin (2) have potent antileishmanial activity, while the non-correlated compounds didn't show any intense activity. The less toxic compound 1, was further evaluated against *L. infantum* intracellular amastigotes, resulting in an EC50 of 2.6 µM, indicating its potential as a promising antileishmanial agent. Overall, the combination of HetCA and STOCSY was able to identify the most active compounds, as demonstrated in this study, facilitating the discovery of bioactive components.

Acknowledgements This work was supported by “GreenCosMIn” project (HORIZON-MSCA-2022-SE-01, Project code: 101131346)

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P417 Development of an identification test for multi-origin herbal medicine *Rhapontici Radix* using HPTLC

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Herbal medicines often originate from multiple botanical sources, yet are frequently distributed under a single name, causing confusion in quality control and standardization.

Rhaponticum uniflorum (L.) DC., *Echinops setifer* L., or *Echinops lactifolius* Tausch (Compositae) in the Korean Herbal Pharmacopoeia (KHP). In contrast, the Chinese Pharmacopoeia (ChP2020) and Hong Kong Chinese Materia Medica Standards (HKCMMS) exclusively recognize *R. uniflorum*. *Echinops lactifolius* as its botanical origin, creating further complexity. Thus, an accurate identification test for *Rhapontici Radix* is required.

Currently, the Korean Herbal Pharmacopoeia (KHP) does not provide an identification test for *Rhapontici Radix*. While the Chinese Pharmacopoeia (ChP2020) does not specify marker compounds for *Rhapontici Radix*, beta-ecdysterone is designated as a marker in the Hong Kong Chinese Materia Medica Standards (HKCMMS). Additionally, alpha-terthiophene is established as a marker for *Echinopsis Radix* in ChP2020. This study aimed to develop an HPTLC analytical method based on pharmacopoeial guidelines, utilizing alpha-terthiophene and beta-ecdysterone to differentiate among the three botanical origins of *Rhapontici Radix* by comparing their HPTLC fingerprints. Additionally, HPTLC-MS was employed to identify a marker compound specific to *Echinops setifer*. The resulting distinct fingerprints enabled clear differentiation among the botanical origins. This study offers a novel identification method for *Rhapontici Radix* and provides foundational data for further research on *Rhapontici Radix*. It also enhances quality control for herbal medicines derived from multiple botanical origins.

P418 (Dis)similarity evaluation of Brazilian “stone-breakers” (Phyllanthae tribe) by NMR-based metabolic profiling

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Several species of the Phyllanthae tribe (Phyllanthaceae) are known as “stone-breakers” and used for their antiurolithic effect in Brazil. This interchangeability is described in reports of popular use, also stimulated by the difficulty of botanical differentiation. However, the scientific and regulatory framework is still lacking indications of this potential use as substitutes. In this controversial scenario, this study aimed to compare the NMR-based metabolic profile of commonly mixed species, namely *Embllica urinaria*, *Moeroris amara*, *M. stipulata*, *M. tenella*, *Phyllanthus carolinensis*, and *P. niruri*. Samples (n = 40) were collected in different cities in Brazil (SisGen AEBEED4). The aerial parts were dried, grounded, and extracted using dynamic maceration (2 x 3h), with ethanol/water (7:3, v/v). The extracts were filtered and the resulting extracts were dried. Each extract was suspended in deuterated methanol and analysed by ¹H NMR. The obtained data was aligned, binned, and scaled for following chemometric analysis. Principal Component Analysis (PCA) allowed to observe different clus-

ters, mainly for *E. urinaria*, *M. amara*, and *M. stipulata*. On the other hand, some overlap was observed between *M. tenella*, *P. carolinensis*, and *P. niruri* samples. By orthogonal projections to latent structures discriminant analysis (OPLS-DA) followed by statistical total correlation spectroscopy (STOCSY), it was possible to indicate potential discriminant compounds between the different species. Even though it is still not possible to predict the impact of the dissimilar chemical profiles on the therapeutic effects, these results highlight that the evaluated species are chemically different and care should be taken to use them as substitutes.

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P419 Development and optimization of a reliable HPLC-MS method for the identification of *Tabernanthe iboga* and its differentiation from toxic look-alikes

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Tabernanthe iboga is a plant containing a broad spectrum of alkaloids, the most significant of which is ibogaine. This alkaloid has been proven effective in the treatment of addiction to various substances, as it alleviates withdrawal symptoms and reduces cravings. Due to these properties, ibogaine clinics have emerged worldwide, incorporating ibogaine into treatment programs. [1, 2] However, the limited availability of authentic *T. iboga* from Africa has led to substitution with other plant species that may contain toxic alkaloids. Reports of fatal cases resulting from such falsification of *T. iboga*, as well as issues related to improper dosing, highlight the need for accurate identification methods. [3, 4] The aim of this study is to enable the differentiation of *T. iboga* from other plants with a high risk of falsification based on their alkaloid profiles, thereby increasing the safety of *T. iboga* use. In this study, an HPLC-MS method for the analysis of 11 iboga alkaloids and their derivatives was developed. Various extraction solvents were tested, with ethyl acetate combined with ammonium hydroxide proving to be the most effective. Real samples from ibogaine clinics were obtained and analysed and the quality control and risks associated with these products were assessed.

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P420 Phytochemical profiling and gut microbiota interactions of *Cannabis flos* extracts: Insights into postbiotic metabolite formation

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Cannabis sativa L. is a medicinal plant with well-documented therapeutic potential, particularly in the management of central nervous system disorders such as epilepsy, chronic pain, and nausea. Its inflorescence (*Cannabis flos*, CF) contains a diverse array of specialized metabolites, including phytocannabinoids and flavonoids, that collectively account for its pharmacological activity [1]. While inhalation remains the predominant route of CF administration, oral delivery through aqueous or hydroethanolic preparations is gaining recognition as advantageous, especially for patients with pulmonary comorbidities, difficulties operating vaporization devices, or requiring prolonged and discreet therapeutic effects [2]. Recent reports underscore the critical role of the gut microbiota in modulating the pharmacokinetics, bioavailability, and efficacy of orally administered natural products. A central aspect of this interaction is the generation of postbiotic metabolites, which may exhibit enhanced or distinct biological activities relative to their precursor compounds [3, 4]. The present study aimed to characterize the phytochemical composition of *Cannabis flos* oral formulations, including infusions and water-ethanolic extracts prepared with or without prior plant material decarboxylation, and to investigate their interactions with human gut microbiota. Qualitative profiling and identification of postbiotic metabolites were performed using high-performance liquid chromatography coupled with diode-array detection and tandem mass spectrometry (HPLC-DAD-MS/MS). The analyses demonstrated the predominance of selected phytocannabinoids and flavonoids, emphasizing the influence of extraction conditions and thermal processing on the phytochemical profile and potential therapeutic properties of CF preparations. Furthermore, chromatographic profiling enabled the detection of several postbiotic metabolites, the biological significance of which warrants further investigation in future studies.

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P421 Development and validation of an HPLC analytical method for preservatives in musk

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Musk has long been recognized and effectively utilized as an animal-derived natural drug in traditional Korean herbal medicine. This study was fulfilled to develop a HPLC method to analyze sorbic acid, dehydroacetic acid, ethylparaben, and methylparaben as contaminants, which have been widely used as food additives but not been known to contain naturally in musk.

All musk samples were obtained from species certified *Moschus moschiferus*, and basically utilized a general test for preservatives in Korean Food Codex. The method validation was done for linearity, LOD, LOQ, accuracy, precision, and system suitability in accordance with MFDS guidelines for pharmaceutical validation.

The findings indicated that LOD varied from 0.13 to 0.36 mg/mL, LOQ ranged from 0.40 to 1.10 mg/mL, and the coefficient of determination (R²) for linear-

ity exceeded 0.99. The recovery rates for four preservatives varied from 73.1 % to 116.8 %, while the precision and system suitability fell within the range of 0.20 % to 0.32 %, satisfying the validation criteria. In addition, none of the four preservatives were detected in 12 musk samples collected from various regions and age [1–3].

The HPLC analytical method developed in this study is expected to improve musk quality control by allowing the detection of deliberately added preservatives.

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P422 Identifying origins of medicinal plant resources by using DNA barcode

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Medicinal plant resources have been widely used as a herbal material in pharmaceuticals, cosmetics, and health functional foods, it is essential to scientifically identify their species for conservation and sustainable use. In accordance with development of analytical techniques, e.g. DNA barcode, a new approach to species authentication makes closely related species authenticate more clearly. In this study, we applied DNA barcoding to develop species authentication method with species-specific genetic markers and analytical conditions of herbal materials authenticated by organoleptic characteristics like appearance, smell, taste and texture [1, 2].

DNA barcoding regions, in chloroplast and nucleus (e. g. ITS) were evaluated for their effectiveness in species authentication. PCR amplification and DNA sequencing has been applied to 53 medicinal plant materials including Licorice (*Glycyrrhizae Radix et Rhizoma*). Comparative analysis was performed to assess genetic variation and species differentiation.

This research showed discrepancies between DNA-based and organoleptic test-based species authentications in some samples. Additionally, intra-species variation was disclosed among genetically matched specimens, highlighting the genetic diversity within certain species. These findings demonstrate the importance of molecular authentication in species authentication and contribute to the systematic conservation and sustainable management of herbal materials, supporting their quality and safety in regulatory science and various industries.

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P423 Mapping olive leaves from Greece with untargeted NMR-based profiling

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Olive leaves, a by-product of the olive oil industry accounting for about 10 % of the total olive crop, are a rich source of bioactive compounds with potential applications in the pharmaceutical and nutraceutical industries [1]. While several studies have been published on the composition of olive leaf extracts focusing on specific compounds [2], there is little information on the phytochemical variability related to environmental and genetic factors [3, 4]. In this study, for the first time, untargeted Nuclear Magnetic Resonance (NMR)-based metabolomic approach combined with HPLC-DAD targeted determination were employed to comprehensively analyze the chemical composition of more than 400 Greek olive leaves samples belonging to different cultivars, collected over different years and geographical areas, and cultivated by different agricultural systems. Multivariate Data Analysis was used to highlight differences among the samples, which were mainly related to oleuropein and its metabolites content, as well as triterpenoids, enabling the classification of samples based on specific biomarkers. While the botanical origin and the year of harvest influenced the expression of the bioactives, the cultivation system seemed to have less impact on the chemical composition. This study demonstrates the effectiveness of NMR-based metabolomics in characterizing olive leaf phytochemistry and provides valuable insights into the chemotaxonomic differentiation of Greek olive cultivars based on unprecedented number of analyzed samples. These findings can be used to create new market opportunities and inform decision making in various industrial and research sectors for olive leaves valorization.

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P424 High Performance Thin Layer Chromatography (HPTLC) fingerprinting for authentication of monofloral honey

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Authenticating the floral origin of honey is a key aspect of quality control, particularly for monofloral honeys, which are derived from a predominant nectar source and commonly yield higher prices than multifloral honeys. While bees

can deliberately target and collect pollen, which serves as their main dietary protein source, they may inadvertently also introduce pollen of their floral nectar source into the nectar during collection. This pollen then becomes a natural honey ‘contaminant’. Melissopalynology, the microscopic analysis of pollen in honey, is a widely used method for verifying monofloral honey on the assumption that honey’s predominant pollen grains align with its nectar origin. However, this authentication technique has its limitations. While it is effective when bees forage from a restricted set of floral sources such as in agricultural settings with controlled pollination, it is limited in its ability to authenticate honeys produced from extrafloral nectaries, pollen-free flowers or those produced in biodiverse environments, where a wide variety of pollen may unintentionally ‘contaminate’ the nectar on its collection [1–3].

Using Jarrah (*Eucalyptus marginata*) honey as a case example, this study highlights the potential of High Performance Thin Layer Chromatography (HPTLC) fingerprinting as an alternative method for monofloral honey authentication in such complex environments. Honey extracts are visualised as sets of HPTLC fingerprints, which capture honey’s typical chemical composition reflective of its nectar origin. The study demonstrates that despite natural variations in composition, the proposed analytical approach delineates typical patterns that can be used to confirm honey’s monofloral status.

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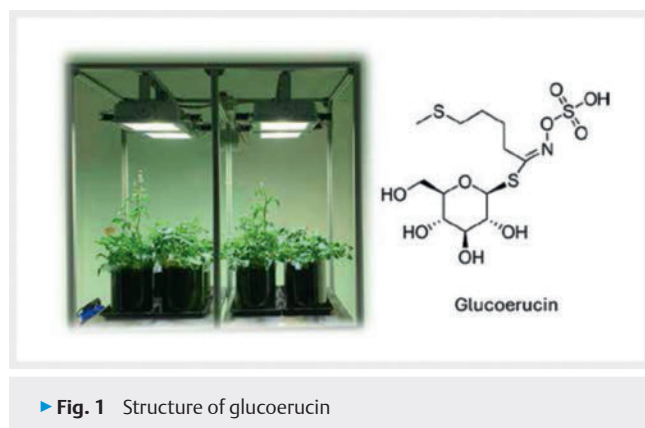
P425 Metabolic profile variation at different growth stages in *Eruca sativa* leaves cultivated in a closed system: Metabolomics by UHPLC-Q-Exactive-MS/MS analysis

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The increasing demand for sustainable, nutritious plant-based products is fostering the development of innovative cultivation systems in the nutraceutical sector. Indoor agriculture, particularly for microgreens, offers an effective approach to producing high-quality biomass with enhanced nutritional value. *Eruca sativa*, commonly known for its high glucosinolate content, is of particular nutraceutical interest due to its potential health benefits, including antioxidant, anti-inflammatory, and anticancer properties [1]. This study investigates the metabolic profile of *Eruca sativa* across four developmental stages—microgreens, vegetative plants, flowering, and fruiting stages—grown in a newly developed closed cultivation system named *Lampada ventilante*. This device recreates a controlled ecosystem using precision lighting and targeted ventilation, enabling pesticide-free growth and consistent yields [2, 3]. Plant material was extracted using Ultrasound-Assisted Extraction (UAE) with ethanol/water (70:30), and analyzed by ultra-high-performance liquid chromatography coupled with high-resolution mass spectrometry (UHPLC-Q-Exactive-MS/MS) in negative ion mode. Data were processed using Compound Discoverer™ 3.3 for metabolite annotation and subjected to Partial Least Squares-Discriminant Analysis (PLS-DA) for statistical interpretation (► Fig. 1).



The results revealed significant metabolic differentiation among the growth stages, with glucosinolates emerging as key markers, particularly abundant in microgreens. Other identified compounds included glycosylated flavonoids, fatty acids, and lipids, showing developmental regulation. This work demonstrates the utility of controlled-environment systems for producing standardized, high-value plant materials while enabling detailed metabolomic studies. The findings contribute to understanding the ontogenetic variation in *E. sativa* and support the integration of microgreens into functional food and nutraceutical formulations.

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P426 Chemical diversity of volatile compounds in *Mentha* species and hybrids across a large germplasm

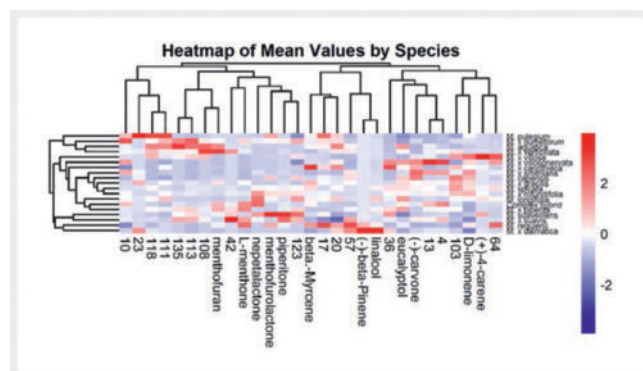
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Mentha species (mints) are economically valuable due to their wide applications in pharmaceuticals, cosmetics, and the food industry [1]. Their essential oils, rich in menthol, carvone, and menthone, are highly valued for their aromatic, therapeutic, and flavoring properties. However, the genus *Mentha* includes 18–30 species and approximately 100 varieties and cultivars, resulting in complex taxonomy and chemical diversity. This study aimed to assess the chemical diversity of 234 *Mentha* accessions, including seven species (*M. spicata*, *M. longifolia*, *M. suaveolens*, *M. aquatica*, *M. capensis*, *M. pulegium*) and 11 hybrids (*M. × piperita*, *M. × rotundifolia*, *M. × gracilis*, *M. × dumetorum*, and others). Volatile Organic Compound (VOC) profiles were generated using headspace solid-phase microextraction coupled with gas chromatography-flame ionization detection (HS-SPME-GC-FID), revealing 29 significant volatile peaks. Boxplot analysis showed notable intraspecific and interspecific variation in VOC composition. Clustering and heatmap analyses identified distinct chemotypic groups and unique biochemical signatures across species and hybrids (► Fig. 1). This study highlights the remarkable chemical diversity within the *Mentha* genus, provid-

ing valuable insights into the genetic and biochemical complexity of *Mentha*. These findings support the sustainable use and conservation of *Mentha* germplasm and can aid in selecting suitable genotypes for pharmaceutical and industrial applications.



► **Fig. 1** Heatmap diagram of 19 *Mentha* species and hybrids with 29 known and unknown compounds in fresh leaves.

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P427 Reversed phase HPLC-DAD profiling of zerumbone in rhizome of *Zingiber ottensii*, extracted with different solvents

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The rhizome of *Zingiber ottensii* Valetton (ZOT) has been traditionally used in Thailand for the treatment of flatulence, wounds, peptic ulcers, and muscle pain. Several studies revealed promising anti-inflammatory and anticancer activities of ZOT extracts, particularly the essential oil and its bioactive compound, zerumbone [1–3]. Recently, we have developed and validated the HPLC method for analysis of zerumbone in ZOT rhizomes [4]. In this study, our experiments were designed to evaluate the HPLC profile of zerumbone in ZOT rhizomes that were steamed (120 °C for 60 min using an autoclave), dried, and then extracted with three different solvents (n-hexane, 95 % ethanol, and distilled water), comparing with non-steamed rhizomes. n-hexane extracts of ZOT rhizomes had two specific peaks, while there were three specific peaks in 95 % ethanol extracts. Among these peaks, zerumbone is a major constituent in both steamed and non-steamed ZOT rhizomes extracted with n-hexane and ethanol, which was eluted at a retention time of 33.4 min. However, it was not detected in the both ZOT rhizomes extracted with distilled water. Under steaming process, the content of zerumbone significantly decreased by about 11 % and 20 % respectively in n-hexane and ethanol extracts of steamed rhizomes compared with non-steamed rhizomes. Besides, it should be noted that n-hexane extracts of steamed and non-steamed ZOT rhizome also contained higher content of zerumbone compared to ethanol extracts. Overall, these results suggested that the steaming process had negative impacts on zerumbone content in ZOT rhizomes, which might affect their biological activities.

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P428 From plant to soil: Investigating pyrrolizidine alkaloid fate using extracts of *Myosotis arvensis* and *Senecio vulgaris*

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Pyrrolizidine alkaloids (PAs) are toxic secondary metabolites found in approximately 3 % of flowering plants and pose potential environmental risks as they enter soil and groundwater. Although their presence in food and pharmaceuticals is strictly regulated in the European Union (EU 2023/915), PA contamination in water remains unregulated. This study investigated the leaching behavior and transformation dynamics of PAs in two soil types common in Latvia (loam and sandy loam), at two depths—10 cm (topsoil) and 20 cm (subsoil)—over a 14-day period. *Myosotis arvensis* (L.) Hill (Boraginaceae) and *Senecio vulgaris* L. (Asteraceae) were used as model PA sources. Cold and hot water extracts from these species were applied to the soils. Leachates were collected, extracted using the QuEChERS method, and analyzed by LC-HRMS.

PAs exhibited distinct leaching patterns influenced by soil type, depth, and chemical structure. *Myosotis* PAs from cold extracts leached more rapidly and were depleted earlier in carbonate-rich loam. In contrast, *Senecio* PAs, particularly cyclic diesters, leached more slowly due to greater chemical stability. PA N-oxides degraded more rapidly than their free base forms, especially in loam, where local conditions promoted transformation. Increased leachate pH in loam was associated with higher PA solubility. In sandy loam, PAs were retained longer in subsoil than in loam. These findings improve understanding of PA environmental fate and support better environmental risk assessments and groundwater protection.

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P429 LC-HRMSMS comprehensive profiling of the polar lipids occurring in the seeds of *Cucurbita pepo* var. *styriaca*

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Cucurbita pepo var. *styriaca* (Cucurbitaceae family) is a pumpkin imported in Europe in the post-Columbian era, still today farmed as a food in almost all warm and temperate areas of the world [1]. The peculiarity of this variety lies in its naked seeds which are used for the preparation of an edible conditioned oil, particularly appreciated for its sensory and optical characteristics, with a recognized use as anti-inflammatory agent [2]. Furthermore, recent studies conducted on seeds hydroalcoholic extracts highlighted their beneficial effects on human health, in particular in the treatment of benign prostatic hyperplasia [3].

Considering that investigations on the chemical composition of the seeds of *Cucurbita pepo* var. *styriaca* were mainly focused on fatty acids composition [2], the present study has been addressed to a broader analysis of the lipid classes, in order to include polar lipids.

The use of liquid chromatography techniques coupled with high-resolution tandem mass spectrometry allowed to identify in the ethanolic extract obtained from pumpkin seeds the occurrence of a high number of polar lipids, mainly phospholipids and sphingolipids (mostly ceramides of the Cer-EOS type, i.e. ceramides in which the fatty acid *N*-acylated to the amino-alcohol long chain base is in turn involved in the *O*-esterification via its ω -OH position with a second fatty acid), and neutral lipids. The obtained results, supported by literature evidences about the potential beneficial roles shown by these metabolite classes for human health, contribute to valorize pumpkin seeds and further encourage their use as a nutraceutical agent.

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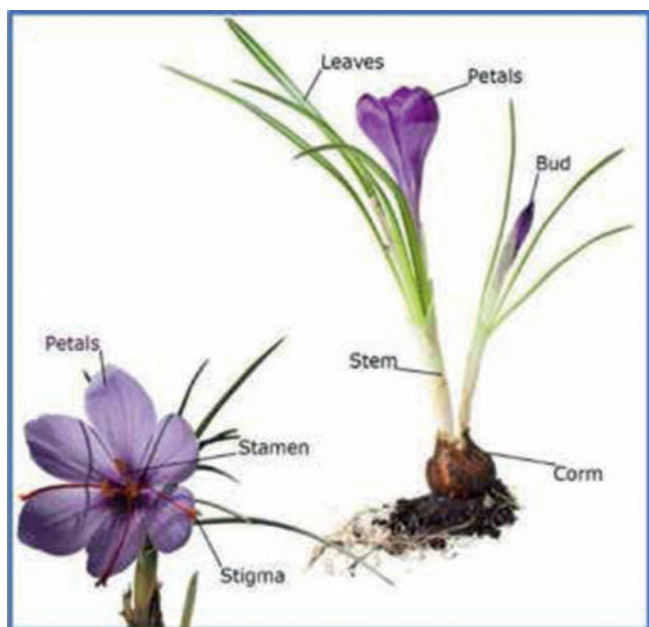
P430 New analytical determinations for the quality of saffron (*Crocus sativus* L.) based on ISO 3632-2011

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Saffron (*Crocus sativus* L.) (► Fig. 1) is a well-known spice cultivated since ancient times for its characteristic yellow-orange color as dye. Because of its high price, saffron is often subject to adulteration. ISO law 3632-1,2:2010/2011 classifies the spice in three commercial categories on the base of absorbance values at 275 nm, 330 nm, and 440 nm [1]. However, this method cannot detect adulterations with additives absorbing at the same wavelengths and showing bands like those of saffron. Therefore, this study aims to replace the ISO-specified spectrophotometric analysis with an HPLC-UV/VIS method that not only detects adulterations but also better separates saffron's key components: crocin, picrocrocin, and safranal [2]. Furthermore, water and moisture content are key parameters for determining the quality of raw materials, ingredients and finished products in the food, pharmaceutical and chemical industries. Moisture and water affect the processability, shelf life, ease of use, quality and cost of raw materials and often the financial margin of finished products. Therefore, it is suggested to use freeze-dried saffron to allow for better preservation and not incur in the typical degradation phenomena of the spice. An accurate measurement of the moisture and water content is a key parameter for controlling production processes and for quality assurance in the food, pharmaceutical and chemical industries. Lastly, to determine the water content of saffron, infrared balance and Karl Fisher titration are used as an alternative to the conventional gravimetric analysis. In conclusion Karl Fischer titration is the technique of choice for water specificity [3].



► Fig. 1 Flower and different parts of *Crocus sativus* L.

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P431 Integrating molecular networking and chemometrics to explore alkaloid diversity in the ethnomedicinally important *Cissampelos capensis* from South Africa

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Cissampelos capensis, the most widely used species in traditional medicine with in South Africa, is valued for its therapeutic properties [1]. However, comprehensive phytochemical profiling has been limited to the use of conventional analytical approaches, creating a knowledge void in understanding its chemical diversity and medicinal potential. This study employed an integrative metabolomics approach combining molecular networking with chemometrics to systematically characterize alkaloid diversity across different plant parts and geographical origins of the species. We investigated phytochemical profiles of *C. capensis* leaves and rhizomes from distinct populations in the Eastern Cape (Maitland, Redhouse, Sardinia Bay, Seaview, Summerstrand) and Western Cape (Atlantis, Bokbaai, Hopefield), South Africa. UPLC-MS data from acid-base extracts (5 mg/mL) were processed using MS-DIAL [2] and analyzed through multivariate statistics (MetaboAnalyst) [3] and feature-based molecular networking (GNPS) [4]. Results revealed diverse alkaloid patterns, with bisbenzylisoquinoline alkaloids predominantly accumulating in rhizomes, while leaves contained diverse structural classes including aporphine, pro-aporphine and benzylisoquinoline alkaloids. Principal component analysis (PCA) showed clear chemotypic differentiation between plant parts (leaves vs. rhizomes) and be-

tween provincial origins. Structural relationships of alkaloids were successfully mapped using the molecular networking technique, offering new insights into the phytochemistry of *C. capensis*. These findings offer valuable data for standardizing traditional medicinal use of different plant parts while establishing a foundation for targeted pharmacological investigations. The study underscores the value of integrative metabolomics in elucidating the chemical complexity of medicinal plants, particularly for understudied species with ethnopharmacological relevance.

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P432 Headspace vs. liquid injection in GC/MS analysis of industrially produced essential oils: A comparative study

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Aromatic plants are a distinct group of species valued for their characteristic scents and flavors, attributed to the production of volatile secondary metabolites. These plants are widely cultivated for their applications in medicine, perfumery, and the food industry. Among their most important products are essential oils (EOs), which are complex mixtures of lipophilic and highly volatile compounds – mainly terpenes – biosynthesized in various plant organs such as leaves, roots, and flowers [1]. Due to the volatile nature of the EOs' constituents, the technique of choice for the analysis of the EOs is gas chromatography coupled with mass spectrometry (GC/MS) [2]. Two of the most common sampling techniques used in gas chromatography are Liquid Injection and Headspace. In Liquid Injection a portion of the sample is directly injected using a microsyringe, while in Headspace GC-MS, the sample is first heated and then the vapor phase (headspace) is collected for analysis [3]. This study aims to investigate the chemical composition of industrially produced essential oils through steam distillation of aromatic plants: lavender, rosemary, basil, thyme, sage, lemon, and chamomile, by GC/MS with Liquid Injection. Additionally, a comparative assessment of Headspace and Liquid Injection sampling techniques was performed on the essential oils to evaluate their efficiency in capturing their volatile profile. Quantitation of major constituents was further performed in both sampling techniques using analytical standards.

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P433 Extraction optimization of active ingredients from *Hypericum perforatum* L. leaves

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Hypericin (HC) and hyperforin (HF) are two bioactive secondary metabolites of *Hypericum* species, widely studied for their antidepressant, antimicrobial, and anti-inflammatory properties [1]. However, their quantification remains challenging due to instability and the lack of standardized extraction and analysis protocols. The present study aimed to optimize an ultrasound-assisted extraction (UAE) process to enhance the recovery of hypericin and hyperforin and to develop an HPLC-DAD method for their detection. UAE of *Hypericum perforatum* L. dried leaves was carried out by testing four solvents (Methanol, Ethanol, Methanol:Ethanol (1:1 v/v) and Methanol:Ethanol:Acetone (1:1:1 v/v)), three extraction temperatures (25, 30 and 40 °C), four timepoints (15, 25, 30 and 45 min), and three solid-to-solvent ratios (1/20, 1/40 and 1/60 mg/mL). For the analysis of the extracts, the HPLC-DAD method development involved modifications of several HPLC parameters [2] – eluents, gradient profile, flow rate, and multiple wavelengths – leading to effective separation and quantification of hypericin at 590 nm [3] and of hyperforin at 270 nm. Optimal extraction of hypericin and hyperforin from leaves was achieved using methanol at 30 °C for 25 minutes with a 1/60 mg/mL solid-to-solvent ratio. HPLC-DAD method validation included intra-day and inter-day repeatability assessments of three replicate optimized extracts. The maximum relative standard deviation of hypericin and hyperforin peak areas of the optimal samples was 1.53 %, confirming the high reproducibility of both extraction and analysis. This optimized method provides a reliable tool for the standardized extraction and quantification of hypericin and hyperforin in plants of the genus *Hypericum*.

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P434 Metabolomics and lipidomics focused on polar lipids of kernel and leaves of *Corylus avellana* cv. Tonda Gentile delle Langhe

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Corylus avellana L. (Betulaceae), commonly known as hazelnut tree, is one of the most popular nut trees worldwide. Hazelnuts are a nutritious food, rich in healthy lipids, extensively used by the confectionery industry. They are consumed either raw (with skin) or roasted (without skin) [1]. Currently, Italy has two hazelnut varieties recognized by the European Union with the Protected Geographical Indication (PGI) label: “Tonda di Giffoni” or “Nocciola di Giffoni” and “Tonda Gentile delle Langhe” or “Nocciola Piemonte” [1]. After an extensive investigation on the different parts (kernels, shells, leaves, flowers, leafy covers) of *C. avellana* cv. Giffoni [1–4], the objective of the present work was to obtain comprehensive information about the metabolome of the kernels and leaves of *C. avellana* cv. Piemonte.

Polar extracts were subjected to liquid chromatography coupled with electrospray ionization and high-resolution mass spectrometry (LC-ESI/LTQOrbitrap/MS/MS).

LC-ESI/HRMS profiling of the leaves revealed mono- and diglycosylated flavonoids as well as diarylheptanoid derivatives previously isolated also from *C. avellana* cv. Giffoni which demonstrated the ability to prevent oxidative damage to human plasma lipids induced by H₂O₂ and H₂O₂/Fe²⁺, exhibiting higher antioxidant activity than curcumin [1, 2].

LC-ESI/HRMS profiling of the kernels confirmed the presence of phenolic compounds found in the leaves, along with a wide range of polar lipids, from oxylipins and long-chain bases (LCBs and LCBPs) to intact high-molecular-weight phospholipids (I-PC/PC, I-PE/PE, I-PI/PI, I-PA/PA, I-PG/PG, PS, and NA-GPEs), sphingolipids (GlcCers), and glycolipids (DGMG, MAG, SQDG).

Based on the biological activities of these polar lipids and their impact on human health, these findings support the inclusion of hazelnuts in human nutrition as a food rich in bioactive and healthy lipids with beneficial effects [5].

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P435 Characterization and composition of seed oil of different grape varieties, obtained by three different extraction methods

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Grape seeds are a valuable by-product of wine production and are increasingly recognized as a source of high quality oil for use in nutrition and cosmetics.

This study compares the efficiency and impact of three extraction methods – supercritical CO₂ extraction, cold pressing and hexane extraction on the yield and composition of grape seed oil extracted from six grape varieties: Pinela, Sauvignon, Pinot blanc, Riesling, Cabernet Sauvignon and Refosco [1, 2]. Supercritical CO₂ extraction yielded the highest oil yield, followed by hexane extraction and cold pressing. Gas chromatography-mass spectrometry (GC-MS) was used to determine the fatty acid profiles of the oils. Linoleic acid was the predominant fatty acid in all samples, followed by oleic acid, stearic acid and palmitic acid. The extraction method had no significant influence on the fatty acid composition, although the content of myristic acid varied between the grape varieties [3].

To evaluate the quality of the oil beyond the fatty acid composition, we quantified the tocopherols and phenolic compounds. High performance liquid chromatography (HPLC) revealed that δ -tocopherols were not present in any sample, while the highest levels of α - and β -tocopherol were found in hexane-extracted oils. The total phenolic content measured with the Folin-Ciocalteu method was also higher in hexane-extracted oils than in cold-pressed oils.

These results suggest that while supercritical CO₂ extraction is optimal for oil yield, hexane extraction improves the recovery of antioxidant compounds, offering potential benefits for therapeutic and cosmetic applications.

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P436 HPLC-DAD method for the quantification of CBD, CBDA, Δ^9 -THC, and Δ^9 -THCA cannabinoids in medicinal formulations

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Cannabis is one of the oldest plants cultivated and contains over 700 characterized compounds, including cannabinoids, with Δ^9 -THC and CBD being the most prominent. The lack of validated analytical methodologies for cannabinoid quantification can result in inaccurate dosages and potential adulteration. High-performance liquid chromatography with diode-array detection (HPLC-DAD) stands out as a robust and accessible tool for quality control. This study aims to develop and validate a methodology for quantifying cannabinoids (CBD, CBDA, Δ^9 -THC and Δ^9 -THCA) in different matrices. Oil samples were provided by legally authorized users. The matrix effect was evaluated to determine the method's selectivity, and a student's *t*-test revealed no significant differences in slopes for the analyzed cannabinoids. Calibration curves ($0.6\text{--}6\text{ }\mu\text{g mL}^{-1}$ for CBD and Δ^9 -THC, and $0.1\text{--}6\text{ }\mu\text{g mL}^{-1}$ for CBDA and Δ^9 -THCA) demonstrated good linearity, with *R*² values ≥ 0.9997 , limits of detection (LODs) ranging from 9.39×10^{-2} to $1.01 \times 10^{-1}\text{ }\mu\text{g mL}^{-1}$, and limits of quantification (LOQs) between 2.48×10^{-1} and $3.07 \times 10^{-1}\text{ }\mu\text{g mL}^{-1}$. The results indicate that the method exhibits high selectivity, linearity, and sensitivity for analyses in different matrices. The absence of matrix effects further supports its reliability and applicability. However, method validation is ongoing, with precision and accuracy evaluations in progress, which are essential to confirm its robustness for routine analytical applications [1, 2].

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P437 Thermostabilization of poppy seeds: Proof and effects

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Varieties of poppy (*Papaver somniferum* L.) with low levels of toxic opium alkaloids are cultivated mainly in Central Europe. Poppy seeds are mainly used in the production of various bakery products. However, waste seeds left from production of pharmaceutical poppy with high morphine and other opium alkaloids content may also enter grey market. Fraudulent producers use thermostabilisation primarily to reduce contamination of opium alkaloids in the seeds and then misdeclare them or their mixture as premium seeds. However, it should be noticed that thermostabilisation is a process primarily intended for inactivation of lipase, enzyme responsible for lipids hydrolysis in damaged or ground seeds. Hydrolytic rancidity, i.e. released free fatty acids cause unpleasant bitter taste of product. In this study, the Fourier-transform infrared spectrometry based method for the proof of poppy seed thermostabilization was applied both to untreated and thermostabilized poppy seeds samples. The developed mathematical model enabled calculation of apparent acid number values. The measurement was performed immediately after grinding the seeds and after 24 hours, respectively. In the case of untreated poppy seeds, the apparent acid number value increases while in opposite to the thermostabilised poppy seeds. To document the effect of thermostabilization on opium alkaloids content in processed seeds, a targeted analysis of 7 major opium alkaloids was performed using HPLC-HRMS. Thermostabilization of poppy seeds showed a significant decrease of the individual alkaloids ranging from 11 to 87 %, with an average loss of 67 % for morphine and codeine for which maximum limits have been recently established.

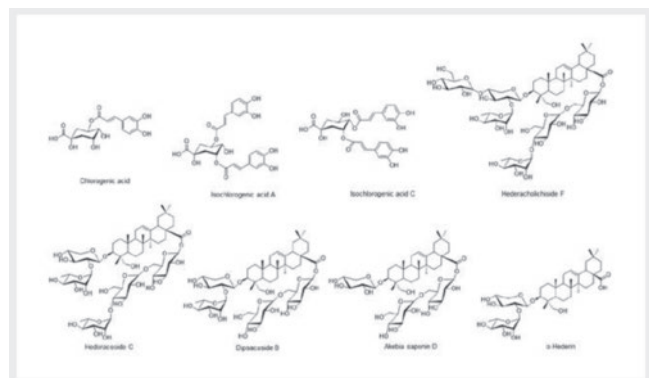
P438 Simultaneous component analysis of *Akebia quinata* seeds by ultra-performance liquid chromatography-tandem mass spectrometry for quality and cytotoxicity assessment title

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Akebia quinata seeds (AQSS) are used as an analgesic, antiphlogistic, and diuretic in traditional herbal medicine [1, 2]. We developed an ultra-performance liquid chromatography with tandem mass spectrometry (UPLC-MS/MS) simultaneous component analysis method to analyze eight compounds (chlorogenic acid, isochlorogenic acid A, isochlorogenic acid C, hederacolchiside F, hederacoside C, dipsacoside B, akebia saponin D, and α -hederin) as markers for the quality assessment of AQSS (► Fig. 1). The separation of the eight analytes was performed in an Acquity UPLC BEH C18 reversed-phase analytical column. The method was validated with respect to linearity (coefficient of determination ≥ 0.994), recovery (90.32–108.18 %; relative standard deviation (RSD) < 10.0 %), and precision (RSD < 10 %). The analysis of the AQSS confirmed that the eight components were found in concentrations of 0.42–9.07 mg/g. The cytotoxicity of the AQSS extract and the eight compounds against human cancer cell lines, including MDA-MB-231 (breast), A549 (lung), HCT 116 (colon), AsPC-1 (pancreas), and A2780 (ovarian), was also assessed, with cisplatin used

as a positive control. In addition, dipsacoside B showed high cytotoxicity in all cell lines. This assay will help to enhance efficacy and clinical research as well as provide a validated quality assessment of AQS extract and related traditional herbal medicines.



► **Fig. 1** Chemical structure of the eight target compounds selected for the quality assessment of the *A. quinata* seeds.

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P439 Optimization of ultrasonic-assisted extraction (UAE) of phenolic and antioxidant compounds from *Ephedra foeminea* Forssk. using response surface methodology (RSM)

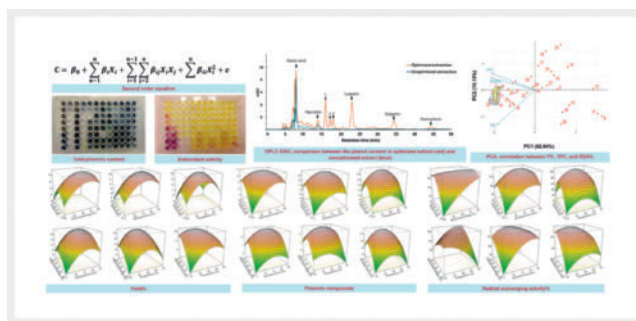
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Ephedra foeminea Forssk., a Mediterranean shrub with diverse traditional medicinal applications, is rich in bioactive metabolites [1, 2]. This study optimized the ultrasonic-assisted extraction (UAE) of phenolic and antioxidant compounds from *E. foeminea* using response surface methodology (RSM). A circumscribed central composite design was applied to assess the influence of four variables—solvent volume (20–40 mL/g), ethanol concentration (50–70 %), sonication time (10–20 min), and temperature (35–45 °C)—on extraction yield (Y %), total phenolic content (TPC), and radical scavenging activity (RSA %). The enhanced statistical models provided the following optimal conditions for each output: Y % (11.93 ± 0.09 %) at V = 28.2 mL/g, E = 67.9 %, S = 19.1 min, T = 39.3 °C; TPC (3.06 ± 0.06 mg GAE/g) at V = 32.6 mL/g, E = 65.1 %, S = 17.3 min, T = 40.5 °C; and RSA % (90.63 ± 1.08 %) at V = 31.8 mL/g, E = 66.7 %, S = 19.7 min, T = 40.5 °C. High-performance liquid chromatography (HPLC) confirmed that the RSM-optimized UAE conditions enriched phenolics, including gallic acid, luteolin, and apigenin [3]. Principal component analysis (PCA) showed strong correlations between TPC and RSA %, with PC1 and PC2 explaining 98.0 % of the variance. These findings establish RSM-optimized UAE as a potent method for extracting

antioxidant-rich phenolics from *E. foeminea*, supporting its potential use in functional and therapeutic applications [4], 5 (► **Fig. 1**).



► **Fig. 1** A graphical abstract of the optimization of ultrasonic-assisted extraction (UAE) of phenolic and antioxidant compounds from *E. foeminea* using response surface methodology (RSM).

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P440 Monitoring fidaxomicin production: an RP-HPLC-UV approach for the quantification and characterization of fermentation broth

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Fidaxomicin, also known as lipiarmycin A3, is a macrocyclic antibiotic used to treat *Clostridioides difficile* infections and is industrially produced by fermentation from various actinomycetes [1]. The complexity of fermentation broths requires a detailed analytical approach to monitor both fidaxomicin production and the presence of structurally related impurities. Identifying these by-products is critical for optimizing fermentation conditions and improving both yield and purity. Due to the structural complexity of fidaxomicin and the presence of by-products with similar molecular structures, only a limited number of HPLC methods have been described in the literature [2]. Therefore, there is a clear need to develop a robust and specific analytical method that ensures batch-to-batch consistency and supports industrial scalability. In this study, we developed an HPLC method to monitor the fermentation process, quantify fidaxomicin, and identify key by-products. This approach provides a valuable tool for process optimization, monitoring, and quality control in the industrial production of fidaxomicin.

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P441 Unveiling the botanical signatures of reunionese honeys through chemical profiling

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This study investigates the classification of Reunionese honeys based on their chemical composition, leveraging the island's unique diversity of landscapes, including coastal zones, tropical forests, volcanic mountains, and varied microclimates ranging from sea level to 3,070 meters. A total of 416 honeys from 100 beekeepers were collected, representing both common varieties (multi-floral, pink pepper, litchi) and rare types derived from endemic species (*Weinmannia tinctoria*, *Coffea arabica* var. *bourbon*, *Labourdonnaisia calophylloides*) and medicinal plants (*Hypericum lanceolatum*, *Hubertia ambavilla*, *Dombeya acutangula*) [1].

Carbohydrate profiling was performed for the first time on Reunionese honey using High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection which identified 17 carbohydrates. [2] Additionally, untargeted LC-MS/MS analysis was conducted on dichloromethane extracts of 82 honey samples representative of the collection.

Untargeted analysis revealed significant clustering of honey samples according to botanical origin, demonstrating clear discrimination. [3] Chemical diversity assessment using molecular networking and Sirius further enabled the annotation of chemical classes and key compound structures. [4, 5] This analytical approach substantiates empirical observations — such as the crystallization capacity — and provides a robust framework for differentiating Reunionese honeys according to their botanical origins.

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P442 Beyond the label: Metabolomic analysis reveals quality variability in commercial *Hyssopus officinalis* essential oils

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Hyssopus officinalis L. (Lamiaceae) essential oils (EOs) are renowned for their complex chemical composition and diverse chemotypes, which hold significant value in traditional and modern medicine, aromatherapy, pharmaceuticals, and as natural preservatives [1, 2]. This study analysed 20 commercial EO samples from the aerial parts of *Hyssopus officinalis* subsp. *officinalis*, sourced from Italy, Spain, France, and Bulgaria. Chemical characterization was conducted using GC-MS-FID and ¹H NMR-based metabolomics, identifying two primary chem-

otypes: eucalyptol and pinocamphones (*cis*- and *trans*-), and a third linalool chemotype. The pinocamphones chemotype was further divided into two groups, one with a high β -phellandrene content. The biological properties of the EOs were evaluated for antifungal activity against *Trichophyton mentagrophytes* and *T. violaceum*, and antioxidant potential using the DPPH test. The pinocamphones chemotype with high β -phellandrene exhibited the highest antioxidant activity (IC₅₀ = 3.24 μ l/ml) and significant antifungal activity, inhibiting *T. mentagrophytes* by 28 % and *T. violaceum* by 15 %. The eucalyptol chemotype showed negligible antifungal activity, except for one sample with 55 % inhibition of *T. mentagrophytes*, distinguished by high aromatic or olefinic compound concentration. Multivariate data analysis provided an overview of the global phytochemical and biological results, emphasizing the need for detailed quality control procedures for commercial EOs, especially given the diverse chemotypes with varying bioactivities and organoleptic properties. This study highlights the potential presence of fraud in the EO market, underscoring the importance of rigorous quality control measures. The predictive OPLS-DA model presented in this study offers a valuable tool for EO quality control.

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P443 A multiplatform approach to diterpenoid profiling in greek *Sideritis* species

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Diterpenoids are particularly widespread across *Sideritis* species and are structurally classified into various subtypes [1]. Despite the prevalence [1, 2], the full extent of diterpenoid chemical diversity within the genus remains underexplored mainly due to structural complexity.

In this study, we present a comprehensive strategy that integrates analytical techniques (LC-MS/MS, GC-MS, and NMR spectroscopy) with computational workflows, including molecular networking and mass defect filtering, to systematically investigate the diterpenoid content of six dichloromethane extracts from Greek *Sideritis* taxa. This combined approach enabled efficient mapping of phytochemical profiles, with a particular emphasis on *ent*-kaurane-type diterpenoids.

Siderol, a well-known *ent*-kaurane diterpenoid, was consistently detected across all samples, reinforcing the role as a characteristic marker in Greek *Sideritis* taxa. Comparative profiling revealed similar diterpenoid composition among *S. euboea*, *S. scardica*, *S. raeseri* subsp. *attica*, and *S. raeseri* subsp. *raeseri*, while *S. clandestina* subsp. *clandestina* and *S. sipylea* displayed more distinct chemical profile. To the best of our knowledge, this is the first study employing a combination of GC-MS, LC-MS, and NMR to profile diterpenoids across *Sideritis* taxa, underlining the value of integrative methodologies in the discovery and classification of bioactive natural products.

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P444 Advancing the quality assessment of natural products with dried spot microsampling: A novel analytical approach

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Efficient analytical workflows are needed for the quality assessment of natural products, including foods, supplements and phytotherapeutics, to promote consumer health. However, the sampling and sample preparation stages remain bottlenecks, demanding resources, time and labor. Dried spot microsampling, predominantly used for dried blood spots (DBS), features minute sample size, easy remote sampling, convenient storage, and transportation via post [1]. The dried spot format also allows for automated setups and is compatible with multiple analytical systems, including LC-MS [2, 3]. Such approaches could provide appealing solutions to the above challenges, yet they remain unjustifiably underexplored in this field.

This work aimed to introduce dried spot methodologies in natural product characterization through proof-of-concept targeted and untargeted applications, using cannabis products and wine, respectively. Robust workflows were developed for chemical profiling of wine and cannabinoid determination in cannabis products (plant material, CBD oils, etc.) through sensitive LC-HRMS/MS analysis, encompassing volumetric sampling on paper cards, designed for field use by non-trained personnel or integration in regulated environments. Important parameters were optimized for throughput, sensitivity and extraction capacity, based on an automated card extraction platform. Through comparative experiments, the proposed methodologies showed similar results to common conventional techniques, while offering convenience, reproducibility, and minimal sample size and handling requirements. Overall, this study presents a novel framework in natural products analysis by unlocking the potential of the dried spot format to foster reliable and resource-efficient quality assessment, amid high-capacity workflows with reduced logistical burdens.

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P445 The relationships between growth and ginsenoside content of wild-simulated ginseng harvested different times

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Wild-simulated ginseng (WSG, *Panax ginseng* C.A. Meyer) should be grown in a natural mountain environment without artificial facilities or chemicals. Therefore, its growth and active compounds content may be affected by various environmental factors. The aim of this study was to investigate the growth and ginsenoside content of 5-year-old WSG harvested monthly for one year. In the analysis of growth characteristics of aerial part, stem length, stem diameter, leaflet length, leaflet width were significantly longer in May~July than in other harvest periods. In the root part, root length and number of rootlets gradually

increased. In the analysis of ginsenoside content in aerial part, F1, F3, F5, mRb1, mRb2, mRc, Rf and Rg2S were significantly higher in April than in other harvesting periods. In the root part, most of the ginsenosides were significantly higher in April. In the correlation analysis, the composition of ginsenosides in aerial part and root part was clearly evident. In addition, the aerial part was clearly differentiated according to harvest time. The root part showed no clear difference, but there was a difference in January and April. The sum of squares of the root part was much lower than that of the aerial part, therefore, it was judged to be low significance. These results can be used as fundamental data to determine the optimal harvest time for WSG and to differentiate it from other ginsengs by analysing the ginsenoside content in the aerial part of WSG [1].

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P446 Chemical investigation of ancient Italian apple varieties to develop a new commercial product using untargeted metabolomic approaches

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Malus domestica Borkh. (Rosaceae) is among the most widely cultivated fruit trees globally, valued for its year-round availability and fruits rich in flavor and health-promoting compounds [1]. These benefits are primarily attributed to its complex chemical composition, including primary metabolites, phenolic compounds, and triterpenic acids [2]. In Italy, a long-standing tradition of apple cultivation has led to the development of numerous ancient varieties with distinctive traits [3]. However, many of these local cultivars remain poorly studied due to limited availability and distribution, despite their potential as sources for new commercial apple products. This study aimed to chemically characterize 24 ancient Italian apple varieties to explore their potential in the development of novel commercial products. Both peel and pulp tissues were analyzed and compared with 10 widely marketed apple cultivars using untargeted metabolomic approaches. Two complementary analytical platforms were employed: ¹H NMR spectroscopy for broad-spectrum metabolite profiling and UHPLC-MS for targeted analysis of phenolic compounds, particularly flavonoids. The resulting datasets were interpreted using multivariate statistical analyses, including principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA), soft independent modeling of class analogy (SIMCA), and hierarchical clustering analysis (HCA). Results revealed that apple peels contained significantly higher levels of flavonoids and phenylpropanoids, whereas the pulps were richer in sugars and organic acids. Clearer differentiation among varieties was observed in peel samples, driven by more abundant phenolic constituents. Several ancient varieties exhibited unique metabolomic profiles distinct from commercial apples, highlighting their potential as sources of health-beneficial compounds for future commercial product development.

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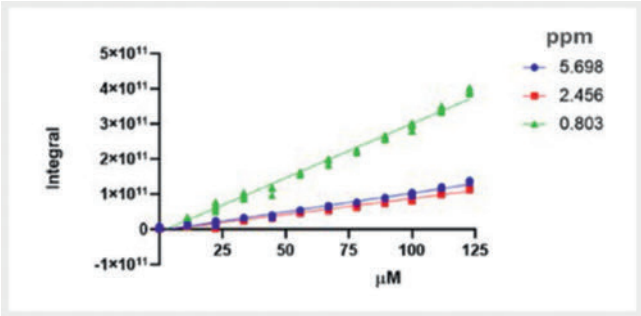
P447 Development of a nuclear magnetic resonance-based quantitative techniques for quality control in herbal medicinal products

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The quality control of herbal medicinal products (HMPs) is still challenging nowadays with their complex composition and inherent natural variability. Typically analysis are based on analytical marker by High-Performance Liquid Chromatography and Gas Chromatography techniques [1, 2]. However, these methods are often time consuming and have high limitation due to high instrument variability. In contrast, Nuclear Magnetic Resonance (NMR) spectroscopy presents a rapid and robust alternative, capable of addressing these limitations [3–5].

The proposed NMR approach enables identification and quantification of target compounds by combined the excellent performance of Bruker IVDr pulse sequences with multiple solvent suppression using shaped pulses. The method was validated in accordance with established EMA guidelines.

Glycyrrhizic acid was selected as the target analytical marker. The NMR spectrum revealed several well-resolved signals corresponding to glycyrrhizic acid (► **Fig. 1**). The method demonstrated excellent linearity, with samples spiked at various concentrations exhibiting a detection limit below 15 µM and a correlation coefficient above 0.99. These results indicate that the method is highly suitable for the quantification of glycyrrhizic acid in herbal products.



► **Fig. 1** Calibration data for Glycyrrhizic acid. Dilution series with 5 independent replicates per concentration. Reproducibility: Mean R2>0.99, RSD = 1.31 %.

NMR spectroscopy provides a fast solution with high robustness for targeted quantitative application in herbal medicine. This technique not only complies with regulatory standards but also enhances the reliability and robustness of quality assessments of HMPs.

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P448 Advancing herbal medicine: Extent similarity assessment for a quantitative fingerprinting approach

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Analysis of Herbal Medicinal Products (HMPs) presents unique challenges due to their complex nature and the often-unknown identity of their active constituents. The guideline of European Medicines Agency (EMA/HMPC/541422/2017 Corr.) requires a comprehensive and robust analytical method. To go beyond the limited scope of established analytical markers, chemometric methods have been applied to evaluate the quality of HMPs [1–4]. Extent Similarity has been underestimated while it is a promising method for the comprehensive quantitative evaluation of fingerprinting data of HMP, especially for HMP categorized as “other extracts” as no active constituents are defined and the whole extract is considered as the active principle, but only proxy analytical markers are monitored. Objective: to develop and validate a quantitative fingerprinting method via HPLC-DAD, specifically utilizing the Extent Similarity method in the quality control of HMPs containing Licorice root fluid extract

The fingerprinting method was applied to an exemplary herbal extract, with a focus on capturing the quality of the mixture in a holistic way. The method involves HPLC-DAD for fingerprint analysis and Extent Similarity method for a standardized quantitative chemometric analysis.

Simulated data (► **Table 1**) mimics a sample diluted to 80 % with minor variations. The results are shown in ► **Table 2**.

► **Table 1** simulated typical liquid chromatographic fingerprinting data of two samples. Sample B is 80 % dilution from Sample A with minor variations in each peak with area below 500.

Simulated Sample	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6
A (reference)	1	10	100	200	500	1000
B	0.7	7.5	80	155	400	800

► **Table 2** Similarity evaluation with different approaches on simulated typical liquid chromatographic fingerprinting data of two samples. Sample B is 80 % dilution from Sample A.

Method data evaluation		Similarity results Vector B
Cosine similarity*	0-1.000	0.98
Correlation Coefficient*	-1.000-1.000	0.99
Extent similarity*	-∞-1.000	0.77
Adapted Similarity Score*	0-1.000	0.87
Euclidean distance**	0-∞	231

* The closer the value to 1, the more similar the vectors are. ** The closer the value to 0, the more similar the vectors are.

The Extent Similarity method can perform a full analysis of an extract like that of licorice root. The fingerprint results assessed by the Extent Similarity method proved to be suitable for the quantitative quality control of HMPs. It demonstrated accuracy, sensitivity, and reliability in capturing the phytochemical constituents holistically.

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P449 From traditional use to assay-guided characterization: phytochemical and biological insights into the potential of *Achillea millefolium*

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Research and characterization of bioactive compounds are becoming key aspects of cosmetics, driven by the increasing importance of plant-based ingredients in formulations. Indeed, the collaboration between academia and industry aims to identify new and high-performance natural actives for innovative cosmetic applications. Among botanical species, yarrow (*Achillea millefolium* L.), a medicinal plant from the Asteraceae family, native to Europe and western Asia and now widespread worldwide, is a prominent candidate for cosmetics applications. With a long history in traditional medicine, in the skin disorders field, yarrow is highly valued for its soothing, anti-inflammatory, wound-healing, skin-lightening, and antimicrobial properties [1]. Our extract, obtained from the aerial parts by rapid solid-liquid dynamic extraction using the Naviglio® extractor [2], was then freeze-dried and the dry extract was used for subsequent chemical and biological analyses. In particular, it was analyzed for

total polyphenol content and antioxidant activity by ABTS analysis, demonstrating antiradical properties. Building on these findings, our investigation focused on *in vitro* assays using keratinocytes (HaCaT cell line), first assessing the extract's safety on cell viability and then evaluating its antioxidant activity through MTT and DCFH-DA assays, respectively. The results showed that, at safe concentrations, the Yarrow extract exhibited a significant scavenging effect, protecting cells from H₂O₂-induced oxidative stress. The next phase aims to investigate its wound-healing properties and protection against stress induced by H₂O₂ and LPS. Finally, we intend to compare these properties with those of a commercially available Yarrow extract, produced using the same matrix and extraction solvent ratio.

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P450 Quality challenges in herbal product registration: Evidence from a five-year Saudi Arabian experience

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The assurance of safety and quality in herbal products is a principal mandate of regulatory authorities. The Saudi Food and Drug Authority (SFDA) mandates the pre-marketing evaluation of herbal products, focusing on the active pharmaceutical ingredient (API) and finished pharmaceutical product (FPP) components [1, 2]. This study aims to identify common quality issues observed during the initial evaluation of herbal products, with the objective of enhancing regulatory compliance and procedural efficiency. A retrospective observational analysis was conducted on herbal product applications submitted to the SFDA between 2019 and 2023. SFDA's internal quality evaluation reports and application data from SFDA systems were utilized. Only applications classified as herbal products were included. Descriptive statistical analyses were performed using SPSS version 26.0. A total of 308 applications were analyzed. Analysis of the API evaluation revealed that 69 % of quality issues were related to product specifications, primarily concerning residual solvents and assay methodology. Additional issues were associated with batch analyses and manufacturing process descriptions. In the FPP evaluation, the highest frequency of quality issues was observed in the Batch Formula and Specifications sections, particularly regarding the completeness of assay data. Furthermore, frequent deficiencies were noted in the labeling of storage conditions and the submission of alcohol-free and pork-free certifications. The quality issues identified during the pre-marketing evaluation of herbal product applications represent a major factor contributing to prolonged registration timelines. To address these challenges, it is recommended that SFDA develop targeted and illustrative guidelines for manufacturers, thereby facilitating timely market access while ensuring the maintenance of rigorous quality standards.

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P451 Safety and efficacy of *Curcuma longa* (turmeric) for joint pain in elderly: A systematic review with regulatory implications

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Joint pain, particularly due to osteoarthritis, is a major cause of disability in the elderly. While nonsteroidal anti-inflammatory drugs (NSAIDs) provide symptomatic relief, their long-term use is associated with serious adverse effects in elderly. *Curcuma longa* (turmeric), a traditional herb, is increasingly marketed as a safer alternative. This systematic review evaluates the clinical evidence on the safety and efficacy of *Curcuma longa* for joint pain relief in the elderly to assist regulatory decisions regarding health claims.

Curcuma longa interventions were included. Outcomes assessed were pain reduction, functional improvement, and adverse events. Twenty studies (15 RCTs, 5 OSs) with ~1,500 elderly participants were included. Most studies focused on knee osteoarthritis and administered 500-1500 mg/day of curcumin over 4-12 weeks. Compared to placebo, *Curcuma longa* significantly reduced pain [mean visual analogue scale (VAS) difference ~18-27 points] and improved physical function [1, 2]. *Curcuma longa* was comparable in efficacy to NSAIDs and better tolerated, with fewer gastrointestinal side effects and no serious adverse events reported [3]. *Curcuma longa* demonstrates moderate efficacy and a favorable safety profile for joint pain management in the elderly. It supports regulatory decisions to include joint pain among accepted claims for Turmeric products.

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P452 Development of callus culture protocol for secondary metabolite production from hemp (*Cannabis sativa* L.)

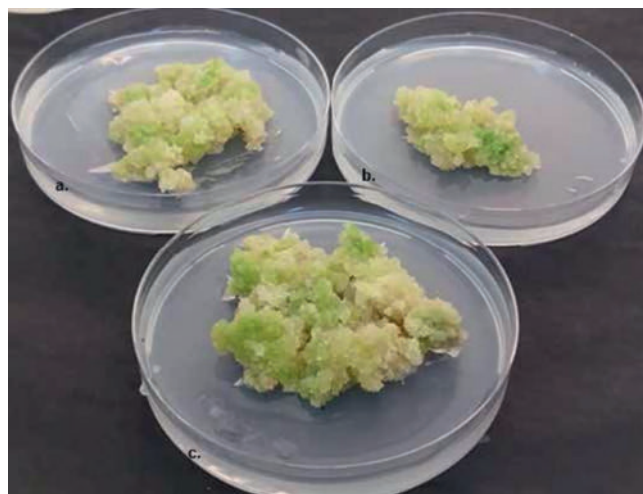
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The hemp contains cannabinoids and other important secondary metabolites of commercial value [1]. Plant tissue culture, which is an alternative soilless, aseptic method for the production of bioactive compounds from plants, is remarkable in terms of providing continuous and more standardized production [2]. In this study, it was aimed to determine the callus induction ability of hemp genotype in media containing different growth regulators (PGRs), to determine the most effective cytokinin-auxin PGR combination on callus biomass production and to determine the subculturing capacity of calli. Three different explant sources; hypocotyl, cotyledon and root were taken and cytokinin [Kinetin, Zeatin, Tiazuron (TDZ) and 6-Benzylaminopurine (BAP)] and auxin [naphthalene acetic acid (NAA)] PGRs were combined at different concentrations and cultured in Murashige and Skoog (MS) nutrient medium. 100% callus formation was observed in all applications and applications were subcul-

tured every 4 weeks. Callus biomasses were recorded between 0.15-12.63 g at 8, 12, 16, 20 and 24 weeks. For each application, the growth indexes of calli at different biomasses (0.5, 1.0, 2.0 g) were calculated in subculturing, and the highest growth index was recorded as 8.58. In TDZ and NAA applications, callus biomass progressed slower compared to other applications, and in the 12th week, approximately 65 % of the applications had callus growth index below 1 and were excluded from the experiment. In the 24th week, callus growth indexes were determined between 0.05-8.44. As a result, the highest yield was determined in cotyledon explants and in the application of Zeatin and NAA PGR (► Fig. 1).



► **Fig. 1** Callus regeneration from cotyledon explants on MS medium supplemented with Zeatin and NAA (Initial fresh weight: a. 1.0 g, b. 0.5 g, c. 2.0 g).

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P453 Pilot trial evaluating *Arrabidaea chica* gel's therapeutic effect on oral mucositis in head and neck cancer patients undergoing treatment

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Oral mucositis is a common side effect in patients with head and neck cancer who require treatments with effective outcomes [1]. The management of this condition remains limited and is primarily palliative [2]. *Arrabidaea chica* demonstrates a safe healing potential by stimulating fibroblast proliferation, enhancing collagen synthesis, and exhibiting antioxidant activity without associated toxicity [3–5]. This randomized controlled trial aimed to evaluate the therapeutic effect of a mucoadhesive gel containing 2.5 % *Arrabidaea chica* extract

in patients diagnosed with oral mucositis resulting from head and neck squamous cell carcinoma, in comparison to treatment with low-intensity laser therapy. The study was designed as a randomized, parallel, open-label clinical trial and was approved by the Research Ethics Committee of the Faculty of Medical Sciences at the University of Campinas (CAAE: 55933516.3.0000.5404). The control group received an 808-nm infrared low-intensity laser in lesion areas during 40 s and the intervention group; treatment received A. *chica* gel three times per day. Mucositis data outcome considered time in days, pain assessment by a visual analogue scale. The control group comprised five participants treated with low-intensity laser, with the intervention group encompassing six participants. The groups treated with laser and 2.5 % A. *chica* gel showed a 2.4 ± 1.9 and 3.3 ± 3.4 ($p = 0.605$) pain scores, respectively. Trial outcome gave for laser and A. *chica* gel an average of 16.4 ± 8.6 and 9.2 ± 3.1 ($p = 0.085$) days to heal. The study was discontinued due to difficulties in recruiting volunteers. The patients only visited the hospital to receive antineoplastic therapy.

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P454 Cranberry food supplements: the quality situation in the real world

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Food supplements often resemble registered drug products in presentation but are not subject to the same strict requirements for efficacy, safety, and quality. Instead, they are regulated under national food laws, resulting in a non-transparent market with frequent quality issues, particularly for supplements that contain botanicals. The present study evaluated the quality of twelve cranberry-based products (*Vaccinium macrocarpon* Aiton, Ericaceae). Samples were obtained from online shops as well as herbal stores and community pharmacies. Cranberry-specific anthocyanins were selected as identity and quality markers and quantified using a validated HPLC method. Moreover, proanthocyanidins (PACs) were quantified through a photometric method based on USP guidelines. Furthermore, HPTLC PAC analysis can confirm the LC and colorimetric data. The analysis revealed serious quality concerns in eleven out of twelve products, indicating adulteration or mislabeling. These findings highlight the urgent need for stricter regulations on food supplements. In particular, developing validated analytical methods to assess the quality of botanical ingredients and intensifying quality control by regulatory authorities are essential to ensure consumer safety.

P455 Quality of food supplements vs. registered herbal drug products from passionflower (*Passiflora incarnata*)

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The aerial parts of *Passiflora incarnata* are used to relieve symptoms of mental stress and promote sleep [1]. In Europe, passion flower products are marketed either as registered herbal medicinal products or as food supplements. However, consumers often struggle to distinguish between these categories. This study analyzed the quality of various passion flower food supplements and registered drug products using validated analytical methods. TLC fingerprinting revealed that five food supplements did not meet specifications, while six products complied (five of which were registered medicinal products). A validated UHPLC method confirmed these findings. LC-MS analysis showed that one food supplement contained only hyperoside, lacking other passion flower-related compounds. Quantitative flavone analysis using photometric and UHPLC methods indicated that five out of six food supplements failed to meet content and identity requirements, suggesting cases of food fraud. In contrast, all registered herbal drug products complied with specifications. As part of the evaluation, product authenticity as *Passiflora*-based supplements was verified and declared flavone contents per unit dose were compared with analytical results to assess labeling compliance [2]. Since the findings align with previous reports on the poor quality of food supplements containing botanicals, increased transparency and stricter quality control measures are recommended. Additionally, manufacturers should conduct routine batch-by-batch analyses, using validated procedures to ensure consistent product quality and consumer safety.

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P456 Optimisation of a green extraction procedure for *Melissa officinalis* L. hydrophilic extract using factorial design

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Melissa officinalis L., commonly known as lemon balm, is a widely studied medicinal plant recognised for its many biological properties, mainly attributed to its essential oil and phenolic compounds [1]. With regard to the polyphenolic fraction a protective effect against UVB-induced damage in human keratinocytes has been reported [2], highlighting its potential for the cosmetic industry. The optimisation of the hydrophilic extract of *M. officinalis* leaves was conducted, focusing on rosmarinic acid (RA) content, using a sustainable extraction procedure.

In detail, an organic solvent-free mixture was employed in combination with Naviglio® Extractor technology, a solid-liquid dynamic extraction process that exploits a pressure gradient to enhance the mechanical transport of bioactive compounds into the solvent [3]. RA, the major phenolic compound in lemon balm, was quantified by UPLC-PAD, and its content was used to optimise three extraction parameters: extractive ratio between drug and solvent (5 % – 7 %), number of extraction cycles (25–30) and solvent composition (a ternary mixture of water/glycerin/butylene glycol in two different relative percentages). A full

factorial design ($2^3 = 8$ trials) was applied to systematically evaluate all parameter combinations.

This approach enabled the assessment of the optimal conditions to maximise RA yield while ensuring an environmentally friendly process. The findings provide valuable insights into green extraction techniques for *M. officinalis*, supporting its added value for cosmetic applications.

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P457 Targeting the gut: How *Chaenomeles japonica*, *Cornus mas*, and *Hippophaë rhamnoides* fruit extracts shape human microbiota *in vitro*?

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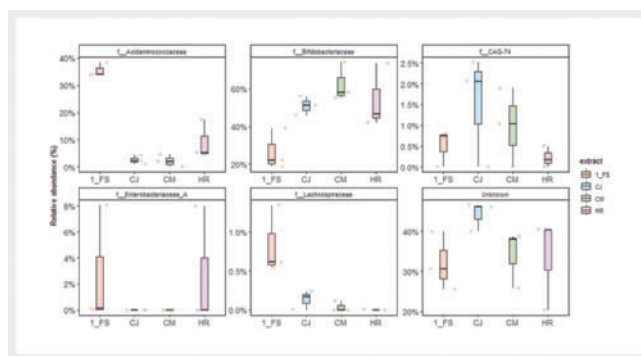
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Dietary foods such as fruits are a potential source of prebiotics, which can help feed the beneficial bacteria in the gut. Medicinal plants and dietary interventions can potentially enhance human health by transforming the gut microbiota [1]. In this study, we raise the question of whether the constituents of aqueous or 70 % hydroethanolic extracts from fruits of *Chaenomeles japonica* (Thunb.) Lindl. ex Spach (CJE), *Cornus mas* L. (CME), and *Hippophaë rhamnoides* L. (HRE) influence gut microbiota *in vitro*.

The study aimed to evaluate α - and β -diversity of the human gut microbiome through next-generation sequencing (NGS) of bacterial 16S rRNA after 24 and 72-hour treatment. A secondary aim was to assess the impact of the extracts on the metabolic activity of both biofilm-associated and planktonic forms of selected bacterial strains, including *Escherichia coli* and *Staphylococcus aureus* [2]. A molecular docking study of the most abundant compounds of the extracts with bacterial enzymes of *Bacillus subtilis* was performed. ATP-dependent helicase/nuclease subunit A and mini-ribonuclease 3 were considered. The preliminary research did not reveal significant changes in gut microbial populations between extracts. Only some changes in the relative abundance of Acidaminococcaceae, Bifidobacteriaceae, and Enterobacteriaceae families were observed (► Fig. 1). CME exhibited *in vitro* inhibition of planktonic cells and biofilm of *E. coli*, while CJE and HRE more efficiently influenced *S. aureus*. The extracts showed a tendency to differentiate gut microbial abundance compared to the control. The absence of statistical significance might underscore the importance of extended exposure times in future studies.



► **Fig. 1** The relative abundance of the top six bacterial families after 72 hours of incubation.

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P458 Anticancer and anti-inflammatory activities of oleoresin and fractions obtained from *Copaifera reticulata* Ducke from Amazon

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Copaiba oleoresin is a natural product widely used in Brazilian traditional medicine, recognized for its anti-inflammatory, healing, antimicrobial, and antitumor properties. Therefore, this study aimed to evaluate the anticancer and anti-inflammatory activities of oleoresin, along with the volatile and resinous fractions of *Copaifera reticulata* Ducke from the Amazon. The oleoresin sample (OB) was collected at FLONA Tapajós in the municipality of Belterra/Pará. The volatile (VF) and resinous (FR) fractions were obtained through hydrodistillation, using both simple distillation and a Clevenger apparatus, and were subsequently fractionated by column chromatography. The chemical composition of the VF was analyzed using gas chromatography coupled with mass spectrometry (GC-MS). The *in vitro* antiproliferative activity was assessed using the sulforhodamine B method in tumor and non-tumor cell lines. Antitumor activity was evaluated in an Ehrlich solid tumor model, while anti-inflammatory activity was assessed using a carrageenan-induced paw edema model. In the VF, 16 compounds were identified, with the major ones being α -bisabolene (30.02 %), α -trans-bergamotene (26.39 %), and α -selinene (10.85 %). OB, VF, FR, and fractions obtained through column chromatography exhibited antiproliferative activity on a panel of human tumor cell lines. OB (75 and 150 mg/kg) and VF (250 mg/kg) significantly inhibited tumor development and relative tumor weight. Additionally, OB (75 mg/kg) and VF (250 mg/kg) demonstrated substantial anti-inflammatory effects on paw edema, affecting both phases of the inflammatory process. The *in vitro* and *in vivo* results confirm the antitumor and anti-inflammatory properties of *C. reticulata* OB, VF, and FR, highlighting their pharmacological potential for developing new anticancer phytopharmaceuticals.

P459 A risk-based approach to contamination screening in botanical food supplements

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The use of botanical supplements is growing, especially in industrialized countries, to support health and well-being. However, safety concerns raised due to contamination with pesticides, heavy metals, mycotoxins, and adulterants, as well as the presence of unintended or deliberately added plant species [1–3]. Therefore, robust quality control measures are essential to ensure product safety not only before but also after commercialization. The variety of available botanical ingredients used to formulate food supplements makes contamination screening a challenging process. Therefore, the selection of relevant plant species based on the market prevalence and potential risk could be beneficial. The present work was aimed at defining risk categories to guide targeted sampling and subsequent analytical evaluation, in the context of a research project funded by the Italian Ministry of Health. A decision-making algorithm was developed to select botanical species based on the following criteria: market diffusion, frequency of occurrence in commercial products, alerts from the Rapid Alert System for Food and Feed (RASFF), reports of suspected adverse reactions from the Italian Phytovigilance System, and available literature on contamination. The algorithm application allowed to create a high-interest list which included *Cynara scolymus*, *Taraxacum officinale*, *Bacopa monnieri*, *Calendula officinalis*, *Curcuma longa*, *Echinacea angustifolia*, *Melissa officinalis*, *Glycyrrhiza glabra*, *Vitis vinifera*, and *Withania somnifera*. Overall, our selection strategy supported the identification of target botanicals for subsequent chemical and molecular analyses, ensuring a representative and risk-informed sampling. This approach provides a model for improving surveillance and quality control in the sector of botanical supplements to ensure consumer safety.

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P460 Chemical, physicochemical and biological characterization of a tocotrienol-rich lipid fraction from *Bixa orellana* L.

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The valorization of industrial plant by-products has emerged as a sustainable alternative for the development of active pharmaceutical ingredients of natural

origin. This study aimed to chemically, physicochemically, and biologically characterize a tocotrienol-enriched lipid fraction (FT3) obtained from the industrial residue production from bixina extraction from *Bixa orellana* L. seeds. Chromatographic, spectrophotometric, thermal, physicochemical, and *in vitro* biological analyses were performed. HPLC revealed average levels of $63.62 \pm 1.34\%$ δ -tocotrienol and $8.00 \pm 0.21\%$ γ -tocotrienol, totaling $71.62 \pm 1.57\%$ tocotrienols. The fraction showed low moisture ($0.11 \pm 0.06\%$), high solid content ($99.89 \pm 0.06\%$), water activity of 0.4783, and dynamic viscosity of 1908 cP at 30 rpm. Thin-layer chromatography confirmed the presence of unsaturated compounds and high levels of terpenes and steroids, whereas phenols, tannins, alkaloids, and coumarins were not detected. FT3 showed antioxidant activity ($EC_{50} = 109.44 \pm 2.32 \mu\text{g/mL}$, DPPH; $0.793 \pm 0.05 \mu\text{mol Fe}^{2+}/\text{g}$, FRAP) and selective antiproliferative effects on tumor lines, notably MCF-7 ($\text{TGI} = 23.5 \pm 19.3 \mu\text{g/mL}$) and U251 ($\text{TGI} = 32.0 \pm 9.3 \mu\text{g/mL}$). The non-tumor HaCaT line also showed sensitivity ($\text{TGI} = 20.2 \pm 0.9 \mu\text{g/mL}$). Accelerated stability testing indicated greater tocotrienol preservation under refrigeration (6.6°C) in aluminum sachets (63.56% after 180 days), while degradation was higher under 45°C in plastic vessels ($59.99 \pm 0.14\%$). FT3 is a promising multifunctional botanical input with potential for cosmetic, foods, and pharmaceutical applications [1,2].

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P461 Assessing the efficacy of medical *Cannabis* as a substitution therapy for opioids in chronic pain management: A comprehensive investigation

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The opioid epidemic has escalated as a result of over prescription, with a 41% jump in usage in 2020. On account of this, medical cannabis has emerged as a possible alternative due to its ability to decrease opiate usage and related deaths.

The study aims to evaluate the effectiveness of medical cannabis in reducing opioid use and related side effects in chronic pain management. Additionally, it seeks to assess improvements in pain relief and quality of life among patients using cannabis as an adjunct therapy. The study employed a quantitative design involving data collection from 302 participants across six South African locations over twelve months. Participants were prescribed medical cannabis and monitored for changes in opioid use, pain levels, and quality of life using questionnaires and pharmacy records. Statistical analyses, including descriptive statistics and linear regression, were utilized to evaluate trends and relationships between variables. Over six months, a study of 302 participants (53.3% female, 69.2% White) found significant reductions in opioid use with medical cannabis. The research findings indicated that 72% of the participants decreased their opioid intake by a minimum of 50%, resulting in an average reduction of 93% (standard deviation = 17%) by the conclusion of the study. The pain severity scores decreased from 3.4 to 3.0, and 64% of participants saw a significant improvement in pain alleviation, which was defined as a reduction of at least two rating points on the pain scale. In addition, 83.2% of the subjects reported experiencing better sleep quality, while 78.5% observed an improvement in physical function. 70.5% of participants indicated a preference for oil as the most effective form of cannabis. This particular form was found to be linked with notable enhancements in pain alleviation and quality of life across many areas.

The study shows that medical cannabis can enhance pain management, sleep quality, and physical function while dramatically lowering the use of opioids. These findings indicate that it has the potential to be a feasible option in pain management approaches.

P462 From hive to formulations: Profiling and quantifying the chemistry of *Scaptotrigona postica* propolis

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Scaptotrigona postica is distributed across several Brazilian states and parts of Bolivia and Peru. A distinctive feature of this stingless bee is its production of propolis, composed mainly of plant resins and bee secretions. The chemical composition of this propolis depends on local flora, influencing its biological properties.

To investigate its chemical profile, propolis samples were analyzed by UHPLC-DAD-QTOF-MS/MS. Chromatographic separation was performed, mass spectra were acquired in positive ionization mode, and data processing was carried out in DataAnalysis and MZmine. Molecular networks (FBMN) were constructed via the GNPS platform and visualized in Cytoscape, yielding .mgf and .csv files with MS/MS spectra and precursor ion data, respectively. Manual inspection and literature comparison enabled the identification of C-flavonoids as the major compounds in *S. postica* propolis. To assess the uniformity of this accumulation, propolis from other stingless bee species from the same location was also analyzed. The integrated molecular network revealed broad flavonoid distribution across species, with most exhibiting flavonoid-O-glycosides. In contrast, *S. postica* propolis was distinctly rich in C-flavonoids, highlighting its unique chemical profile and potential pharmaceutical relevance.

Given this distinctive composition and its promising properties for cosmetic applications, topical formulations for skin and hair were developed using *S. postica* propolis. A targeted MRM-based analytical method was established to quantify key marker compounds in the formulations. The results showed that the active constituents remained stable, supporting the potential use of this propolis in cosmetic and personal care products.

P463 Secondary metabolites from Turkish endemic plant *Scrophularia mesopotamica* Boiss

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The genus *Scrophularia* (Scrophulariaceae), has more than 300 species of perennial herbs found in Asia, Europe, and North America [1]. It comprises ca 78 taxa in Türkiye [2]. Various *Scrophularia* species were employed in traditional medicine to address a range of dermatological disorders, constipation, neuritis, and inflammatory bacterial-viral diseases, including pharyngitis and laryngitis, as well as hyperthermia. Phytochemical assessments have demonstrated that *Scrophularia* species are characterized mainly with iridoids, and phenylpropanoid derivatives [3]. Due to the limited phytochemical research on one of the Turkish endemic *Scrophularia* species; *S. mesopotamica* is selected for further phytochemical investigation. Aerial parts of the plant were extracted with aqueous methanol, and then partitioned between hexane, chloroform, ethyl acetate, and n-butanol, respectively. n-butanol extract was selected for further isolation studies; as a result of targeted LCMS analyzes which were applied to identify the phenolic profile of the both crude methanol and sub extracts with the help of the in-house compound library that consist of ca 100 phenolic compounds from different classes. Repetitive chromatographic methods led to the isolation of flavonoids, iridoids, and phenylpropanoid derivatives from n-butanol extract. Structures of the isolated compounds were elucidated by 1D, 2D NMR experiments, HRESIMS analyzes together with the comparison

of the spectral data with the relevant literature. This is the first report on the isolation of these compounds from *S. mesopotamica*.

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P464 Non-targeted metabolomic studies on regeneratively grown *Calendula officinalis*

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The cultivation of *Calendula officinalis* under regenerative organic agricultural practices represents a promising approach for sustainable phytochemical production. This study presents non-targeted metabolomic analyses of lipophilic extracts obtained from regeneratively grown *Calendula officinalis*. The metabolomic profile was assessed using Ultra Performance Liquid Chromatography coupled with High-Resolution Mass Spectrometry (UPLC-HRMS), identifying 76 bioactive compounds spanning triterpenoids, flavonoids, and polyphenols. The lipophilic extract exhibited a total triterpene content of 1.5 % (w/w oil) as determined by the vanillin-sulfuric acid method, indicating a significant phytochemical contribution beyond the sunflower oil solvent. Antioxidant capacity (ABTS assay) revealed a notable Trolox Equivalent value of 2.51 mmol/L* ▶ **Table 1.**), highlighting the extract's potential bioactivity. The study also monitored extract stability over eight months, oleolites remained visually stable across conditions. These findings support the role of regenerative farming in enhancing secondary metabolite accumulation in medicinal plants and reinforce *Calendula officinalis* as a valuable botanical for cosmeceutical applications [1–5]. Future work will focus on comparing conventional and regenerative crops and refining extraction protocols and bioactivity validation in cosmetic formulations.

▶ **Table 1** Summary of Metabolomic and Stability Findings for *Calendula officinalis* Extracts

Parameter	Lipophilic Extract (Oleolite)
Extract Composition	Sunflower oil infusion
Metabolomic Analysis	UPLC-HRMS identified 76 compounds identified: polyphenols, flavonoids, triterpenoids, fatty acids
Total Triterpene Content	1.5 % (w/w oil)
Antioxidant Capacity (ABTS assay)	2.51 mmol Trolox Equivalents/L
Stability (1-8 months)	Stable color, minor scent variations at 45 °C
Solubility Considerations	Requires liquid-liquid extraction for analysis

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P465 Phytochemical stability and antioxidant synergy of *Myrtus communis* in the Longevity Complex™ for cosmetic applications

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The development of high-performance botanical ingredients is essential for advancing modern skincare formulations. This study focused on the extract of *Myrtus communis*, commonly known as myrtle, a Mediterranean plant traditionally used for its antioxidant, soothing, and antimicrobial properties [1–3]. Several experiments were conducted employing Naviglio® dynamic solid-liquid extraction on dried myrtle berries. Among these, we present a comparative analysis of two specific trials with different solvent systems: Trial A (propylene glycol-based, lower preservative concentration) and Trial F (butylene glycol-based, higher preservative concentration).

The evaluation of free radical scavenging activity with the ABTS assay, along with the quantification of specific flavonol glycosides, revealed a higher yield of bioactive molecules in the butylene glycol-based system. The *Myrtus communis* extract with the highest active compound content was combined with *Spinacia oleracea* leaves extract and *Tephrosia purpurea* seed extract to formulate the Longevity Complex™, aiming to enhance synergistic antioxidant activity. This effect was assessed through *in vitro* assays using fibroblast cultures derived from donors of three different age groups, under exposome stress conditions including UV radiation, pollution, and cortisol exposure. The active ingredient complex demonstrated a potent reduction in reactive oxygen species (ROS) across all tested age groups, showing a decrease in ROS content by 30–32% when compared with positive control.

This study highlights the pivotal role of solvent and preservative systems in influencing the bioactive compounds yield. Moreover, the strategic combination of natural active ingredients promotes synergistic efficacy, as evidenced by the antioxidant activity of the Longevity Complex™.

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P466 *Achillea millefolium* and the science of regeneration: A botanical case study in sustainable innovation

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Achillea millefolium L. (Yarrow) is one of the most revered medicinal plants in the Western herbal tradition, with documented use dating back to antiquity. Traditionally applied for wound healing, anti-inflammatory, and astringent purposes, the herb has attracted growing scientific interest for its phytochemical richness, including apigenin, quercetin, and chlorogenic acid [1]. At Davines Group, *A. millefolium* has been cultivated using regenerative organic methods at the European Regenerative Organic Center (EROC), and incorporated into two flagship product launches: the *Sublime Skin* line and the *We Sustain Beauty* solid shampoo bar. These applications demonstrate the plant's multifunctionality in addressing oxidative stress and sensitive skin conditions, while also reflecting Davines' commitment to ethical sourcing and sustainability [2].

Furthering this commitment, Davines has adopted *Achillea* through the American Botanical Council's Adopt-an-Herb program and is collaborating with Roy Upton and the American Herbal Pharmacopoeia to aid with completion of the official botanical monograph [3]. This initiative not only supports conservation and education, but also positions Davines as a scientific authority on yarrow in the global botanical community.

This presentation outlines a full-spectrum approach to botanical innovation—from cultivation and phytochemistry to formulation and education—framing *Achillea millefolium* as a symbol of modern pharmacognosy in action.

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P467 Supporting the protective functions of the lower female reproductive tract with tannin-rich botanicals

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The lower female reproductive tract (vagina and ectocervix) is inhabited by a unique microbiota, mainly composed of *Lactobacilli*, which adhere to the epithelium, forming a protective barrier. Vaginal dysbiosis causing inflammatory response of epithelial cells, affects up to 60% of women and significantly increases the risk of STIs and preterm birth. In traditional medicine, tannin-rich botanicals have been used to treat vaginal infections and inflammation. Nevertheless, their efficacy has yet to be evaluated.

The study involved the preparation of 70% ethanolic extracts of *Lythri herba* (LH) and *Quercus cortex* (QC), followed by their phytochemical analysis using UHPLC-DAD-MSⁿ. Subsequently, the effects of the extracts on the viability and

inflammatory response of vaginal epithelial cells were evaluated. Cell viability was assessed using the MTT assay after a 24-hour incubation with extract concentrations ranging from 15.6 to 1000 µg/mL. The extracts did not exert a negative effect up to 50 µg/mL, with IC₅₀ values of 552.31 ± 59.23 µg/mL for QC and 596.89 ± 61.76 µg/mL for LH. The anti-inflammatory effects were studied on TNF-α-stimulated VK2/E6E7 cells (10 ng/mL). Both extracts significantly inhibited the secretion of chemokine 8, with IC₅₀ values of 20.32 ± 5.94 µg/mL (QC) and 19.19 ± 7.62 µg/mL (LH). *Quercus cortex* and *Lythri herba* extracts have been shown to be safe for vaginal epithelial cells and to exhibit potent anti-inflammatory properties, thus making them promising agents in alleviating symptoms of vaginal dysbiosis.

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P468 Development of flavonoid analysis from mulberry using High Performance Liquid Chromatography determination

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Flavonoids from plant species of genus *Morus* (commonly known as mulberry) are famous for their antioxidant potential. The genus *Morus* contains about 68 species and the majority of them are cultivated in Thailand [1]. A high performance liquid chromatographic (HPLC) method was developed and validated for quantitative determination of flavonoids (rutin, catechin and quercetin) in the extract of mulberry leaf (*Morus alba* Linn.). The analysis was carried out on a reversed-phase column (C18 with 150 × 4.6 mm) using methanol and 0.1 % formic acid as mobile phase under gradient system. The flow rate was 1.0 mL/min and the detection for rutin was at 280 nm, catechin and quercetin was at 360 nm. The validation method for all standards presented a linear correlation coefficient = 1.000. Relative accuracy for all standards was 90–110 % in the concentration range of 25–125 µg/mL. The accuracy showed percent recovery was in the range of 70–110 %. The Intermediate Precision showed Percent Relative standard deviation (%RSD) for rutin, catechin and quercetin were 2.56, 2.38 and 1.93, respectively. The limit of detection (LOD) for rutin, catechin and quercetin were 0.074, 0.069 and 0.065, respectively. The average percent recovery in the extract of mulberry leaf of rutin, catechin and quercetin were 95.49, 90.17 and 92.26, respectively. Results obtained in this study will serve as quality control and useful reference for the determination of flavonoids in the other samples.

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P469 *Harpagophytum procubens*: Ex-situ propagation and amplified quality production

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Harpagophytum, a medicinal plant native to the Kalahari desert, has become a popular alternative medicine in Europe. The plant is now a recognized medicine. Once gathered by the local Bushmen to relieve various symptoms, it is now harvested and exported on a large scale to Europe. There have been several reports of a serious decline in populations of this species due to unsustainable harvesting. Our study therefore aims to demonstrate the possibility of producing *Harpagophytum* tubers without harvesting in endemic areas.

An in vitro *Harpagophytum* culture trial was undertaken. After decontamination, the seeds were placed on a Murashige and Skoog [1] medium (MS). After germination, propagation tests of the germinated plants (70 genotypes) were carried out on different MS media containing different concentrations of growth regulators such as BAP and ANA. The rooting of all the genotypes was initiated on MS 1/2 medium supplemented with 0.5 mg/l of IBA in order to continue our study with ex-vitro acclimatization and the growth of potted plants in greenhouses. An initial one-year culture trial in 1-litre pots was carried out to select genotypes that regularly produced at least one primary tuber. The following year, a 2-year greenhouse growth trial was carried out with 14 selected genotypes. The plants were removed from the pots and the number of primary and secondary tubers was measured. The collected tubers were dried and analyzed for harpagoside content. As a result, clonal propagation, acclimatization, and off-ground cultivation produced *Harpagophytum* tubers with harpagoside content exceeding European standards, reaching up to 57 %.

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P470 Valuing the extinct species *Sophora toromiro*: in vitro callus cultures as source potential of prenylated flavonoids for cosmetics

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Sophora toromiro is an endemic from the Easter Island. It is classified as extinct in the wild since 1994, but some individuals survive in botanical and private collections. Here, we describe for the first time a promising and efficient in vitro protocol for conservation purposes through micropropagation of *S. toromiro* from seeds obtained from the Viña del Mar National Botanical Garden, Chile. Whilst *Sophora flavescens* and *S. japonica* are currently widely used in cosmetics for its biological activities, *S. toromiro* is unknown in the sector. Literature survey highlights the potential of *S. toromiro* as a valuable source of flavonoids particularly isoflavonoids and prenylated isoflavonoids. The latter show a wide range of biological activities, such as anti-cancer, anti-inflammatory, neuroprotective, anti-diabetic etc...and are highly demanded in cosmetics. In addition, for the development of an extract derived from *S. toromiro* cell culture, an INCI proposed by the Personal Care Products Council (PCPC) could comply with IECI standards, thereby guaranteeing its international recognition and acceptability. To exploit these capacities, we have successfully induced callus development of *S. toromiro* from in vitro stems. Efforts on callus proliferation and homogeneity are currently being tested, altering concentrations of 2,4-D, in combination with BAP and active charcoal (AC). We aim to obtain culture suspensions allowing a continuous and homogeneous production of metabolites of interest from *S. toromiro*. The results presented here greatly contribute to the value for this species regarding its potential as a source of phytochemicals with prospective cosmetic benefits through innovative production pathways.

P471 Innovative herbal formulation as monotherapy to treat topically onychomycosis: An observational retrospective study

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Onychomycosis is a fungal infection of the nail that affects approximately 5 % of the population worldwide. The causative agents are usually dermatophytes and less often yeasts and non-dermatophyte molds (NDMs). The main problems

of onychomycosis treatment are the long time needed to obtain a complete cure, the resistance to drugs, occurrence of adverse effects and the high relapse rates [1, 2]. The aim of this study was to investigate the effect of an innovative herbal product on onychomycosis. From 2017 to 2024, 28 patients (7 males, 21 females, age range: 14–83 years), with onychomycosis caused by dermatophytes [*Trichophyton rubrum* (17)], yeasts [*Candida albicans* (3), *C. parapsilosis* (1), *C. krusei* (1)] and NDMs [*Aspergillus fumigatus* (2), *A. niger* (1), *A. flavus* (1), *Scopulariopsis brevicaulis* (1)], were treated topically twice a day with an oleolite containing *Salvia rosmarinus* Spenn. extract, cinnamaldehyde, carvacrol, thymol and eugenol [3]. Except 2 subjects [*A. fumigatus* (1) and *A. niger* (1)] everyone else achieved a complete cure, namely elimination of the infective fungal organism (mycological cure) and restoration of the normal appearance of the nail (clinical cure). The mean time in months to complete therapy was 5.4 for dermatophytes, 8.7 for yeasts and 5 for NDMs. No adverse effects were noticed. The disease-free patients were very satisfied by the treatment. No relapses were observed during the follow-up. The described natural remedy showed an excellent efficiency and safety without problems of resistance development, therefore might provide an alternative as monotherapy or addition to current options for treating onychomycosis.

Conflicts of interest P. Kallimanis has a patent that covers the synthesis of the herbal product (Kallonychos®, CPNP No 4848065).

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P472 Treating mild to severe plaque psoriasis topically with *Salvia fruticosa* Mill. rich in carnosic acid: An observational retrospective study

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Plaque psoriasis (PqP) is a lifelong skin disease requiring long-term therapy. The aim of this study was to investigate the efficacy and safety of an innovative herbal O/W cream (Kallm.) containing *S. fruticosa* Mill. leaf extract rich in carnosic acid [1] in patients with mild to very severe PqP. To our knowledge, in the worldwide literature such study has been reported only by our team. From 2004 to 2024, 57 patients (33 males, 24 females, age range: 3–75 years) used the Kallm., applying it to psoriatic lesions and around them, twice daily, for at least 2 months. No concurrent therapies were used. 26 out of 57 patients (45%) experienced a complete clearance (100% improvement) of their disease [mean PASI (Psoriasis Area Severity Index) initially = 9.9, range 0.6–26.6], the mean time to achieve this result was 4 months, 28/57 patients (49%) improved from a mean value of PASI = 6.5 to PASI = 2.7, while the remaining 3 presented an aggravation from a mean PASI = 6.4 to PASI = 8.3. The follow up (max 15 years) showed that the clear state lasted from 2 months to 5 years. However, when relapses occurred, patients responded to retreatment in less time than initially. No adverse effects were observed. We concluded that *S. fruticosa* rich in carnosic acid is a useful agent for topical monotherapy of PqP, with high efficacy, able to induce disease clearance and achieving long lasting remission without side effects. Moreover, it was shown to be safe and effective when treating relapses.

Conflicts of interest P. Kallimanis has a patent that covers the synthesis of the herbal product (Kallm.®, CPNP No 4922966).

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P473 The African Herbal Pharmacopoeia 2 – towards evidence based product development

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The African Herbal Pharmacopoeia (AfHP) was first published in 2010 by the Association of African Medicinal Plant Standards (AAMPS). This pioneering work aimed to establish quality control standards for 52 key African plants used in traditional and modern medicine. It also sought to promote trade in African herbal medicines by providing a reliable reference for their safety and efficacy. The publication was widely praised and served as a foundation for further research and development in the field of African medicinal plants [1].

In 2025, a new edition of the AfHP was released, marking a significant evolution from its predecessor. Unlike earlier regional or national botanical checklists, the 2025 edition goes beyond cataloging plants to include comprehensive information on their phytochemical, pharmacological and toxicological properties. This shift reflects the growing emphasis on ensuring the safety, efficacy, and quality of herbal medicines, especially as their global market expands.

The updated AfHP incorporates detailed monographs for numerous plants, covering aspects such as botanical descriptions, phytochemical constituents, ethnopharmacological uses, toxicity profiles, and trade information. It also addresses critical issues of identification and standardization which are key for regulating ingredients and products in the herbal medicinal products (HMP) industry.

Furthermore, the 2025 AfHP aims to support efforts to integrate traditional medicine into national healthcare systems across Africa. It supports the development of an African pharmaceutical industry while safeguarding biodiversity and protecting against biopiracy. By providing standardized guidelines for research, manufacturing, and regulation, the AfHP aims to enhance the credibility and global competitiveness of African herbal products.

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P474 Investigation of the total proanthocyanidin content, and polymerization pattern in the leaves of 12 *Salix* species during the growing season

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Salicylic alcohol derivatives (SADs), flavonoids, and proanthocyanidins (PAs) are the main compound classes in *Salix* [1]. Even though SADs play an important role in the anti-inflammatory efficacy, other phenolic compounds, like PAs, also contribute to these effects [2, 3].

This study investigated the leaves of 12 *Salix* species (*Salix aurita* L., *S. bicolor* Ehrh. ex Willd. (synonym: *S. phylicifolia* L.), *S. caesia* Vill., *S. caprea* L., *S. cinerea* L., *S. daphnoides* Vill., *S. fragilis* L., *S. hastata* L., *S. lapponum* L., *S. purpurea* L., *S. viminalis* L., and *S. ×sepalcalis* Simonk.) with 42 individuals during the growing season of 2018 and 2019. These samples were analyzed for their total PA content, oligomer (DP2–DP10) and polymer content, and the PA pattern using a UPLC®-Diol-FLR method [4]. *S. caprea* (14.6–17.7 mg/g) and *S. daphnoides* (12.2–19.4 mg/g) contained the highest total PA contents whereas *S. bicolor* (1.6–2.2 mg/g) showed the lowest. Of the 12 species examined, *S. hastata* (68 ± 26% polymers) and *S. caesia* (57 ± 26% polymers) constitute an exception because of a lower percentage of oligomers in the total PA content compared to the

polymer fraction. Regarding the PA pattern, the content increased during the growth period within each degree of polymerization (DP), decreased towards the end, and declined with increasing degrees of polymerization. However, this trend did not continue with the polymer fraction.

The present results demonstrate the interspecific and interseasonal variability of the PA content and composition which could have an impact on the harvest time.

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P475 *In vitro* vs. *in vivo* studies on the interaction of natural products with the gut microbiota: the example of STW 5

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The gut microbiota is increasingly seen to be a key factor in the therapeutic action of natural products. This is especially the case in functional diseases with a multifactorial etiology, as e.g. Irritable Bowel Syndrome (IBS) and functional dyspepsia (FD). Given the raising numbers of microbiota studies in preclinical models, it is a matter of current discussion, whether *in vitro* models well predict the *in vivo* situation. The objective of this study is to compare *in vitro* to *in vivo* data for a medicinal product with clinically proven therapeutic efficacy in IBS and FD, STW 5. A comprehensive literature review was conducted using PubMed and other scientific databases to collate data on STW 5's impact on the gut microbiome at phyla and genera levels. *In vitro* studies demonstrated an increase in *Enterococcus* and Firmicutes and a decrease in *Clostridium*, *Bacteroides*, *Blautia* and Proteobacteria, with no change in *Lactobacillus* [1, 2]. *In vivo* studies in Wistar rat models of a healthy GI tract, UC and FD showed minor increases in Firmicutes, Bacteroidetes, *Lactobacillus* and *Blautia* and a significant decrease e.g. in Actinobacteria, *Bacteroides*, *Bifidobacterium*, and *Prevotella*, dependent from the model [3–5]. The different responses of some genera like *Blautia* and *Enterococcus* to STW 5 in *in vitro* models as compared to *in vivo* models highlight the complexity of its interactions with the microbiota, underscoring its multi-target mechanism of action. The identification of consistent microbial shifts associated with STW 5 provides mechanistic insights in its clinically proven health benefits.

P476 Environmental influence on the growth and pharmacologically active compounds of *Eleutherococcus senticosus* stems in Korean cultivation sites

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Eleutherococcus sessiliflorus (Rupr. & Maxim.) S.Y.Hu (Araliaceae), native to East Asia, has been traditionally used for alleviating rheumatic symptoms, enhancing musculoskeletal strength, supporting hepatic and adrenal functions, and reduce fluid retention [1, 2]. These traditional uses are attributed to its anti-in-

flammatory, adaptogenic, and diuretic properties [3]. A total of 104 individuals were sampled from 26 cultivation sites across South Korea. Stem and soil samples were collected in triplicate from each site. Growth characteristics were measured, and meteorological data were obtained from the Korea Meteorological Administration. Quantitative analysis of chlorogenic acid and Eleutheroside E was performed using a validated UPLC method. Climatic conditions, particularly sunlight exposure and temperature, were significantly correlated with plant growth. Interestingly, while stem diameter tended to decrease in more fertile soils, plant height increased, suggesting a trade-off in biomass allocation. Eleutheroside E content was positively correlated with both sunlight duration and stem fresh weight. These findings highlight the critical role of environmental factors, such as soil properties and regional climate, in influencing both the growth and pharmacological quality of *E. sessiliflorus* [4, 5].

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P477 Plant oils for dermal applications: Fatty acid composition determines pro-regenerative effects on skin cells *in vitro*

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Keratinocytes and fibroblasts play fundamental roles in skin function, contributing to normal physiological processes, tissue repair and overall skin health. While plant oils are commonly used in dermal applications, their specific effects on skin cells remain incompletely understood [1, 2].

This study examined the influence of selected plant oils, fatty acids and unsaponifiable compounds on the proliferation and migration of human keratinocytes and fibroblasts *in vitro*. Oils were selected based on their specific fatty acid profiles, including coconut, olive, linden, poppy, pomegranate, marigold and linseed oils [3]. Cell proliferation and migration were assessed using MTS and gap closure assays.

Our results showed that the tested biological activity of the oils depended primarily on the fatty acid composition of their triglycerides. Oils rich in essential fatty acids, particularly linoleic acid, significantly promoted cell proliferation. In contrast, unsaponifiable compounds showed no effect, while punicic acid and pomegranate seed oil had a significant negative impact. The oils did not exert notable effects on the closure rate of the artificial wound gap; however, sterculic and oleic acids inhibited gap closure and induced changes in cell morphology.

These findings support the role of plant oils and their composition in enhancing skin cell regeneration, and provide *in vitro* evidence to support their application in cosmetic and dermatological formulations aimed at skin repair and evidence-based skincare.

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P478 Comparison of the antibacterial and antiadhesive effects of *Tormentillae rhizoma* ethanolic extract and its gut metabolites against enterotoxigenic *E. coli*

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Enterotoxigenic *Escherichia coli* (ETEC) is a public health threat, causing diarrhea, dehydration, and malnutrition, particularly in developing countries. Its virulence factors disrupt gut homeostasis, promoting inflammation, dysbiosis, and increased permeability, contributing to conditions such as inflammatory bowel disease, irritable bowel syndrome, and leaky gut syndrome. The growing problem of antibiotic resistance highlights the need for alternative therapies. *Tormentillae rhizoma* ethanolic extract (TRET) has been traditionally used for gastrointestinal disorders, but its bioactivity may be altered by gut microbiota metabolism [1, 2]. This study aimed to investigate the antibacterial and antiadhesive properties of TRET and its gut metabolites against ETEC. The antibacterial activity of TRET and its gut metabolites was evaluated *in vitro*, in a post-biotic environment, and using an *ex vivo* gut microbiota model. The effects on ETEC adhesion were assessed using an *in vitro* intestinal epithelial model (Caco-2 cell line). TRET at 5 mg/mL inhibited ETEC growth, while lower concentrations and metabolites exhibited bacteriostatic effects. Additionally, the metabolites significantly reduced ETEC adhesion to the intestinal epithelium. The results indicate that TRET, which previously showed a beneficial impact on microbiota biodiversity, may help manage ETEC infections, provided that its concentration is carefully adjusted to maintain a balance between bacteriostatic, antiadhesive, and potential cytotoxic properties.

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P479 The combined use of *Patriniae Herba* and FOLFOX in colon cancer treatment – a preclinical integrative medicine study

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Patriniae Herba (PH) is commonly prescribed by Chinese medicine practitioners to colorectal cancer (CRC) patients, thus the concurrent use of PH with chemotherapeutics of CRC is likely to occur. Although our previous studies [1, 2] have demonstrated anti-tumor effects of PH aqueous extract (PHW) in colon cancer animal models, there is no scientific evidence to justify the therapeutic benefit of its combined use with chemotherapeutics, nor unwanted herb-drug interaction reported. Hence, this study aimed to evaluate the combined use of PHW and FOLFOX (5-fluorouracil, folinic acid and oxaliplatin), one of the first-line chemotherapy regimen for CRC, in terms of the anti-tumor efficacy and the metabolism of FOLFOX component drugs, in preclinical models.

Nude mice bearing human HCT116 colon xenograft were treated with FOLFOX and/or PHW for three weeks, with tumors and blood collected at end-point for analysis. The levels of metabolizing enzymes/molecules of FOLFOX component drugs in blood were also determined. Results showed that the combined PHW and FOLFOX treatments exerted stronger inhibitory effect on tumor growth, when compared with PHW or FOLFOX alone treatments. Besides, oral administration of PHW did not significantly alter the plasma levels of dihydropyrimidine dehydrogenase, serine hydroxymethyltransferase and cysteine, in colon xenograft-bearing mice, indicating that PHW would not interfere the metabolism of FOLFOX component drugs.

In conclusion, the beneficial potentials and the safety of combined use of PHW and FOLFOX have been scientifically verified, which justified their combination use as an integrative medicine approach in colon cancer management.

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P480 Safety and physiological effects of granodiorite as a feed additive for enhancing quality and muscle and fat composition in Korean beef

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This study investigated a functional material with granodiorite, a natural inorganic substance, as its effective ingredient, aiming to establish an academic foundation for the development of feed additives that improve the quality of Korean beef and enhance muscle and fat composition through dietary supplementation [1]. The toxicity, safety, and physiological activities were confirmed through *in vitro* and *in vivo* mechanism studies [2]. To evaluate the toxicity of granodiorite, acute, 4-week repeated, and 13-week repeated oral toxicity tests were conducted on rats, revealing no macroscopic abnormalities such as mortality, general symptoms, weight changes, or autopsy findings. No granodiorite-related changes were observed in hematological and histopathological examinations, and differences from the excipient control group or physiological normal range were minimal, indicating no toxic changes. *In vitro* results showed increased expression levels of PPAR γ , C/EBP α , and fatty acid synthase-related proteins and genes that promote adipogenesis during adipocyte

differentiation. Additionally, it improved fat formation by suppressing the expression level of β -catenin, a negative regulator of fat formation. It decreased catenin expression by lowering the p-GSK3 level in Wnt- β -catenin signaling. These results suggest that granodiorite improves fat formation by inhibiting the expression of Wnt- β -catenin. The findings of this study provide scientific evidence for granodiorite as a functional material for feed additives and can establish a foundation for the future value of lipid resources.

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P481 The impact of Pt and Cu nanoparticles on the production of bioactive compounds and elements – study in *in vitro* culture model of *Brassica oleracea* var. *acephala* (kale)

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The study focused on the biotechnological and phytochemical investigation of *in vitro* cultures of *Brassica oleracea* L. var. *acephala* (green kale), a species from the Brassicaceae family. The research aimed to optimize shoot culture conditions and evaluate the effects of supplementing Murashige and Skoog medium (without plant growth regulators) with non-ionic nanoparticles (NPs) of platinum (PtNPs) and copper (CuNPs) at concentrations of 5, 10, and 15 ppm (0 ppm as control). The study assessed the impact of these NPs on polyphenolic compounds, glucosinolate profiles, and elemental bioaccumulation. Polyphenolic analysis (HPLC-DAD) identified protocatechuic, chlorogenic, caffeic, sinapic, ferulic, and rosmarinic acids, as well as flavonoids quercetin and kaempferol, in all extracts. The most significant enhancement of phenolic compound production was observed with 15 ppm PtNPs, leading to a 618 % increase in rosmarinic acid content compared to the control. Elemental bioaccumulation analysis (ICP-MS) revealed a concentration-dependent uptake of supplemented NPs, with the highest accumulation (396.45 ppm Pt) detected in shoots treated with 15 ppm PtNPs. Additionally, glucosinolate profiling (UHPLC-HRMS) identified 12 glucosinolates in extracts supplemented with 5 ppm PtNPs, including progoitrin, glucoiberberin, gluconasturtiin, and neoglucobrassicin. These findings highlight the potential of nanoparticle supplementation in modulating bioactive compound production in plant tissue cultures, offering innovative applications for biotechnology and functional food industries.

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P482 Skin aging offset: searching for active ingredients in extracts from *Cistus* L. species

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The Paleolithic rock art sites in Côa Valley (Portugal), a UNESCO heritage site since 1998, are surrounded by numerous plants of medicinal and cultural value. Among them, *Cistus albidus* L. (CA), *Cistus ladanifer* L. subsp. *ladanifer* (CL) and *Cistus salviifolius* L. (CS), have been explored as sources of natural ingredients [1]. Their ethnopharmacological relevance to skin diseases prompted the authors to investigate their potential for skin anti-aging applications [1, 2]. Hydroethanolic extracts were prepared and characterized through High-performance liquid chromatography-photodiode array detection-electrospray ionization tandem mass spectrometry (HPLC-DAD-ESI/MS), revealing the presence of ellagic derivatives in all extracts, as well as myricetin and quercetin derivatives in CS and CA. At non-toxic concentrations (CL: 1.0 mg/mL; CS: 0.4 mg/mL; CA: 0.2 mg/mL), cytoprotective effects were observed in normal human dermal fibroblasts (NHDF) when submitted to *tert*-butyl hydroperoxide (*t*-BHP; 0.5 mM). However, only CS and CL exhibited significant antioxidant activity in these cells ($p < 0.001$ and $p < 0.0001$ versus *t*-BHP, respectively) when measured using the 2',7'-dichlorodihydrofluorescein diacetate (H2DCFDA) assay. Also, only CL demonstrated significant anti-inflammatory potential in LPS-stimulated RAW 264.7 macrophages ($p < 0.0001$ versus LPS). A series of enzymatic inhibition assays were performed, including the evaluation of anti-hyaluronidase, anti-tyrosinase and anti-elastase effects, confirming the pronounced anti-aging properties of CS. Ultimately, the Organization for Economic Co-operation and Development (OECD) Test Guideline No. 439 confirmed that CS and CL extracts do not exhibit irritant effects. The skin anti-aging potential of CS and CL extracts underscores the need for further investigation into the development of plant-based skincare products.

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P483 Crosstalk between natural products from *Symphytum officinale* root, skin cells, and human skin microbiota

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Comfrey root (*Symphytum officinale*) is a medicinal plant traditionally valued for its potent anti-inflammatory properties [1]. Due to its rich chemical composition and therapeutic benefits, comfrey root is a key ingredient in many popular ointments, creams, and tinctures that are applied directly to the skin. These formulations are commonly used to alleviate muscle pain, joint inflammation, and minor injuries [2].

This research aims to verify the effects of 70 % ethanol comfrey root extract on skin microbiome diversity, its metabolism by skin microbiome, and the anti-inflammatory impact of its compounds and possible metabolites on keratinocytes and fibroblasts.

16s rDNA sequencing showed that the tested extract (at 2 mg/ml) has no influence on the biodiversity of the skin microbiota after 24 h incubation. However, some qualitative changes in microbiota composition were observed. Changes in extract composition after incubation with skin microbiota for 24, 48, and 72 h were also assessed, although new metabolites were not identified. The ELISA assay showed an increased production of IL-6 and a decreased production of IL-8 in keratinocytes, while both IL-6 and IL-8 levels decreased in fibroblasts.

The results showed extract alters the composition of skin microbiota. That may play a role in extracts' activity in the treatment of skin diseases. Microbiota-enhanced biodegradation of extracts was detected. The anti-inflammatory effect of comfrey root extract was confirmed.

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Conflict of interest The authors declare no conflict of interest.

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P484 Isolation and characterization of exosome-like nanoparticles and their evaluation as agents/ carriers in functional cosmetics

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Exosomes are extracellular vesicles with a size range of 50–500 nm, composed of a lipid bilayer membrane and secreted by cells [1]. They transport lipids, nucleic acids, proteins and cellular metabolites, playing a fundamental role in cell-to-cell communication. The term exosome-like refers to their plant-derived nature. These exosome-like nanoparticles (ELNs) can be isolated from different edible plants, such as grapefruit, cabbage, carrot, broccoli, lemon, ginger, etc.. Due to their small size and natural origin, ELNs are considered biocompatible and safe, making them promising carriers for bioactive compound delivery.

Moreover, they exhibit intrinsic bioactive properties, including anti-inflammation, anticancer and antioxidation effects, which underline their potential applications in both pharmaceutical and cosmetic fields, ELNs can be exploited to encapsulate active ingredients, such as peptides, enhancing skin penetration and protecting sensitive compounds from degradation [2]. Indeed, when incorporated into creams, serums and other topical formulations, ELNs can address major skin concerns like aging, pigmentation, and oxidative stress-induced environmental damage [3].

Building on these premises, this project aims firstly to identify the optimal plant matrix, considering different plant parts, developmental stages, and stress conditions, then the isolation and characterization of these exosome-like nanoparticles using different techniques. The next phase involves *in vitro* experiments on keratinocytes to evaluate biocompatibility, cellular uptake and eventually biological effects. Finally, ELNs will be loaded with active cosmetic compounds, integrated into a cosmetic formulation, and assessed for efficacy.

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P485 Untargeted investigation of *Silybum* spp.: exploring the specialized metabolome beyond silymarin

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The genus *Silybum* (tribe Cardueae, family Asteraceae) currently includes two species, *S. marianum* (L.) Gaertn (milk thistle), well-known for its use in folk medicine, and the less common *S. eburneum* Coss. & Durieu [1]. The achenes of both species accumulate high amounts of a mixture of flavonolignans derived from the oxidative coupling of taxifolin and coniferyl alcohol, collectively known as silymarin [2]. The strong hepatoprotective properties of silymarin [3] have focused phytochemical research in these taxa almost exclusively on flavonolignans, and on the mechanisms regulating their synthesis and accumulation [4]. Notably, exhaustive characterizations for other components of the specialized metabolome of *Silybum* spp. are still scarce.

In this study, we employed an untargeted metabolomics approach to characterize and compare the specialized metabolomes of two genotypes of *S. marianum* (leaves and achenes) and one genotype of *S. eburneum* (achenes). The UPLC-qTOF-MS analysis of the methanolic extracts revealed expected differences in silymarin profiles [5], and specific accumulation patterns for several metabolite classes. In the achenes, these included uncommon derivatives of hydroxycinnamoylquinic acids, lignanamides, and glycosides and dehydrated forms of flavonolignan isomers. Interestingly, two sulfated glycosides of syringic acid and two hydroxycinnamoylamides were exclusively found in *S. eburneum*. In the leaves of *S. marianum*, mainly characterized by luteolin and apigenin glucuronides, chlorogenic and dicaffeoylquinic acids, no silymarin flavonolignans were detected. Nonetheless, the presence of various metabolites possessing the guaiacyl (e.g., pinoresinol glycosides) and syringil moieties suggests a selective use of the monolignol precursors in *S. marianum*, primarily directed toward the synthesis of flavonolignan in the achenes and other lignans/neolignans in the leaves.

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P486 Pharmacokinetics of casticin 3'-glucuronide in a phase I trial in healthy volunteers after administration of different dosages of *Vitex agnus-castus* BNO 1095

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Vitex agnus-castus L., fructus has a long history of medicinal use in the treatment of gynaecological disorders like premenstrual syndrome, irregular menstruation and mastodynia [1]. However, pharmacokinetics of VAC constituents in humans have not been studied so far; even in animals only scarce data is available. A high-resolution LC-ToF-MS bioavailability screening in rat plasma identified casticin-3'-glucuronide as one of the most intense signals detected after oral administration of VAC dry extract. This phase II metabolite of the flavone casticin – itself representing a major constituent of VAC [2] – was therefore selected as marker substance to study the pharmacokinetics of VAC in a single center, open-label phase I clinical trial (EudraCT number 2022-003480-20). Healthy volunteers (n = 36; average age 31.4 years, 24 female/12 male) received 8 single oral doses of 80, 40 or 20 mg VAC BNO 1095 on 8 consecutive days. Plasma concentrations of casticin-3'-glucuronide were quantified by HPLC-MS/MS following protein precipitation and further sample clean-up using Ostro™ plates (Waters). The quantitation method was fully validated according to the ICH M10 guideline and provided accurate and precise results down to an LLOQ of 0.1 ng/ml. AUC values of casticin-3'-glucuronide were found to be dose proportional and likewise, Cmax concentrations increased with ascending dosages. Accumulating effects were observed after multiple dosing on day 8. This is the first study reporting bioavailability and pharmacokinetic data for VAC in humans. Furthermore, safety and tolerability were confirmed for VAC dosages even 4x higher compared to the marketed product Agnucaston® 20 mg.

Conflicts of interest All authors are employees of Bionorica research GmbH and Bionorica SE, respectively. Bionorica SE is manufacturer of Agnucaston® and Agnucaston® 20 mg.

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P487 Plant-derived fructans for cosmetic innovation: Functional characterization of inulin with varying degrees of polymerization in hair smoothing applications

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The global transition toward sustainable cosmetics has amplified interest in plant-derived alternatives to synthetic ingredients such as silicones, particularly in haircare applications. Inulin, a fructan-type polysaccharide extracted from *Cichorium intybus* (chicory), presents notable film-forming and conditioning properties, offering potential benefits for frizz-prone and textured hair. This study investigated the performance of inulin across three degrees of polymerization (DP: n = 4, 8 < n < 13, and n > 22), applied as 0.5 % aqueous solutions to standardized curly/frizzy hair tresses. Samples were subjected to controlled environmental conditions (25 °C, 60 % RH), and compared to a benchmark commercial smoothing mask containing silicones. In line with existing evidence on polymer behavior in cosmetic emulsions [3], high-DP inulin (n > 22) exhibited superior film-forming capacity, resulting in enhanced hair softness, manageability, and shine. Sensory evaluation involving 15 panelists indicated a marked preference for the inulin-treated samples over the silicone-based control. These findings support the application of high-molecular-weight inulin as a biodegradable, effective alternative to synthetic conditioning agents, emphasizing the value of functional biopolymers in the development of sustainable personal care formulations [1, 2], Table ► 1.

► **Table 1** Comparative performance of chicory-derived inulin grades in hair conditioning

Inulin Grade (DP)	Film-forming Performance	Sensory Score (0-10)	Notes
n = 4	Low	4.2	Weak structure
8 < n < 13	Moderate	6.7	Noticeable improvement
n > 22	High	8.9	Superior performance, soft feel

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P488 Antispasmodic effect of a herbal preparation on small intestine: Potential therapeutic benefits for IBS patients

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Amara-Drops contains nine individual herbal extracts and is traditionally used to treat gastrointestinal dysmotility. It has been shown that the extract of Amara-Drops (Amara extract) targets the muscarinic receptor M2, inducing relaxation of carbachol-contracted guinea pig fundus strips, potentially improving

impaired fundus accommodation in functional dyspepsia patients [1]. However, its antispasmodic properties to improve visceral hypersensitivity, a major factor in abdominal pain experienced by Irritable Bowel Syndrome (IBS) patients, have yet to be studied. While anticholinergic agents are commonly used to treat IBS, they often have broad side effects. Herbal medicines have demonstrated excellent antispasmodic properties without significant adverse reactions [2]. Therefore, we investigated the antispasmodic effect of Amara extract on guinea pig small intestine with or without carbachol-induced contraction. Ileum muscle strips were prepared and set up in organ baths in a longitudinal orientation. Exposure of Amara extract (94.4–1,000 µg/mL) to muscle strips induced significant relaxation at 450 µg/mL (25 %, $p < 0.01$), with stronger effects observed at 650, 850, and 1000 µg/mL (27–31 %, $p < 0.0001$), showing a generally stronger relaxation than the positive control isoprenaline (9.8–18 %, $p < 0.05$ – $p < 0.001$). Significant relaxation was also observed in carbachol-contracted muscle strips already at 283.3 µg/mL (50 %, $p < 0.01$), with stronger effects at higher concentrations (78–132 %, 450–1000 µg/mL, $p < 0.0001$) compared to isoprenaline, which only showed significant relaxation at the highest concentration (71 %, $p < 0.05$).

These data highlight the potential of Amara-Drops in modulating visceral hypersensitivity and abdominal pain in IBS patients by inhibiting cholinergic-induced contraction in the small intestine.

Conflict of interest All authors are employees of Weleda AG.

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P489 Potential of Amara extract, a preparation of nine herbal extracts, in alleviating opioid-induced constipation

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Opioids such as morphine and codeine are analgesic drugs, but their therapeutic value is limited due to side effects, particularly opioid-induced constipation (OIC) [1]. Morphine inhibits intestinal motility by targeting μ -opioid receptors [2]. While some μ -opioid receptor antagonists treat OIC, they often cause adverse effects like diarrhea [3]. Herbal remedies have shown promise in alleviating OIC symptom. Previous studies have demonstrated that the extract of Amara-Drops (Amara extract), a preparation containing nine herbal extracts traditionally used for gastrointestinal dysmotility, binds and inhibits μ -opioid receptors (IC_{50} : 109 and 202 µg/mL, respectively). Further research was needed to explore its therapeutic potential in OIC *in vivo*. Studies were conducted in ICR mice, with Amara extract (400 mg/kg) or vehicle administered by oral gavage (PO) 30 minutes before morphine (10 mg/kg) or vehicle. At 60 minutes post-morphine, the animals received a PO suspension of 5 % charcoal in 10 % gum Arabic solution and were sacrificed 15 minutes later. The intestines were removed, and the extent of charcoal movement was measured. Morphine significantly ($p < 0.05$) inhibited intestinal transit (IT) in the charcoal meal test compared to the vehicle control (33.8 % and 48.4 %, respectively). Amara extract itself did not effect the IT at 60 or 90 minutes before charcoal meal administration (45.1 % and 48.6 %, respectively). Notably, Amara extract significantly ($p < 0.05$) mitigated the morphine-induced inhibitory effect and increase IT (41.6 %). These findings highlight the potential of Amara-Drops in alleviating symptoms of OIC. Further studies are needed to determine whether Amara extract can also reduce impaired motility following morphine treatment.

Conflict of interest All authors are employees of Weleda AG.

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P490 Anti-inflammatory properties of Hepatodoron®, a herbal mixture of *Fragaria vesca* and *Vitis vinifera*, in treating liver inflammation

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Hepatodoron® is traditionally used to treat impaired liver function, including stimulation of liver activity and chronic inflammatory disorders. It is a mixture of wild strawberry leaves (*Fragaria vesca*, Fv) and grapevine (*Vitis vinifera*, Vv). Liver inflammation, often caused by viral infections, alcohol abuse, or fatty liver disease, is a common early stage in many types of liver disease. This study investigated the anti-inflammatory properties of Hepatodoron® and its individual plant components. The samples used were plant powders of Fv and Vv, as well as the mixture of those ones used in the final product (Hepatodoron mixture, Hm). Inflammation is typically characterized by the release of pro-inflammatory leukotrienes, prostaglandins, cytokines, and activation of the NF- κ B pathway. We analyzed the inhibition of pro-inflammatory enzymes 5-LOX and COX-2 using recombinant human enzymes. NF- κ B activation was studied in a reporter assay using the human Jurkat cell line. Hm and its individual plants strongly inhibited COX-2 enzyme activity in a concentration-dependent manner (IC_{50} : 3.5, 3.2, and 3.6 µg/mL, respectively). 5-LO enzyme activity was also inhibited, with Fv showing the strongest effect, followed by Hm and Vv (IC_{50} : 8.48, 25.8, and 35.8 µg/mL, respectively). Additionally, all three showed inhibitory effects on NF- κ B reporter activity, with Vv showing the strongest activity (IC_{50} : 264.7, 369.8 (Fv), and 370.9 (Hm) µg/mL). These data suggest that the Hepatodoron mixture is an effective treatment option for patients suffering from liver inflammation due to its potent anti-inflammatory properties. Further studies are ongoing to characterize the potential therapeutic advantages of Hepatodoron®.

Conflict of interest All authors are employees of Weleda AG.

P491 Optimisation of the extraction methods of biologically active compounds from plants used to support nervous system function

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Civilization-related diseases, including neurodegenerative disorders, represent a growing medical and socioeconomic burden in developed societies. Age-related decline in neurocognitive functions intensifies interest in preventive strategies and complementary therapies based on natural bioactive compounds. Plant-derived substances, particularly polyphenols, exhibit diverse health-promoting properties, including antioxidant activity, and may support pharmacological treatments in nervous system disorders. This study aimed to compare extraction techniques accessible to non-specialists (infusions, two decoction types, and macerates) and optimize their conditions (temperature, duration) to maximize the yield and stability of active constituents. Based on literature and medical consultations ten medicinal plant species (*Bacopa monnieri*, *Centella asiatica*, *Gynostemma pentaphyllum*, *Humulus lupulus*, *Melissa of-*

ficinalis, *Ocimum tenuiflorum*, *Rhodiola rosea*, *Salvia officinalis*, *Scutellaria baicalensis*, *Valeriana officinalis*), free from interactions with synthetic drugs and commonly used in neurological health support, were selected for the research. Extracts obtained from them *via* various methods were analysed for polyphenol content and multifunctional antioxidant capacities (tests verifying free radical scavenging – ABTS, reducing properties – FRAP, and chelating ability – FeMICA were performed). Results demonstrated that the type of herbs material (*i.a.* herba, flos, semen, radix, rhizoma) significantly determines the optimal extraction method. Although variation exists across species, elevated temperatures generally enhanced polyphenol contents and the extracts' reducing properties and radical-scavenging abilities, while simultaneously diminishing their metal-chelating potential. Obtained findings underscore the critical role of extraction method selection – especially for average users – in optimizing the therapeutic potential of herbal preparations. The study supports the ergonomic use of traditional extraction techniques to enhance the efficacy of phytotherapy aimed at supporting the well-being of the nervous system. Detailed results and species-specific recommendations will be presented during the accompanying poster session.

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P492 Comparison of the extraction efficiency of phytochemicals from plants used to support respiratory system function

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The COVID-19 pandemic has contributed to long-term complications and general population-level weakening of the respiratory system. This situation encourages the search for natural preventive, regenerative methods to support standard therapies used for respiratory system disorders. Herbs have long been recognized as sources of health-promoting compounds. Given that many disorders involve secondary oxidative stress, the antioxidant properties of phytochemicals are especially valuable. The present study compared different extraction methods (infusions, two types of decoctions, and macerates) to optimize their parameters to maximize yield and efficacy of phytochemicals in extracts. Based on a literature review and medical consultations, ten species of safe plants (with no known interactions with conventional drugs) traditionally used in the prevention and supportive treatment of respiratory diseases were selected (*Althaea officinalis*, *Ammi visnaga*, *Plantago lanceolata*, *Pulmonaria officinalis*, *Thymus serpyllum*, *Thymus vulgaris*, *Tussilago farfara*, *Verbascum densiflorum*, *Veronica officinalis*, *Viola tricolor*). In the obtained extracts, total polyphenols were assayed, and multidirectional antioxidant ability was assessed using ABTS, FRAP, and FeMICA tests. Results indicated that the choice of optimal extraction method largely depends on the types of herbal material used. The optimal technique variants varied among the plant species studied, but it was generally observed that decoction was found to promote higher polyphenol content, enhance the reducing power, and improve free radical scavenging properties. However, high-temperature methods significantly reduced the chelating activity of the extracts. Full results and recommendations will be presented in a poster session. The research purposefully focused on simple, average user-accessible extraction methods to maximize the therapeutic potential of herbal raw materials used by patients with respiratory system disorders.

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P493 Antibacterial activity and phytochemical characterisation of aerial parts of *Cedrus atlantica*

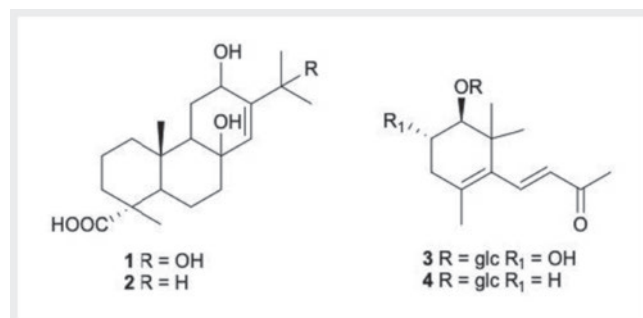
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Cedrus atlantica (Endl.) G.Manetti ex Carrière is a species endemic to the mountains of North Africa. It's a coniferous tree that tolerates drought and cold and can grow on clay and loam soils, highly estimated for its technological, ecological, and biogeographical values [1]. In particular, its essential oils are known for their antifungal, antimicrobial, antiviral, and anti-inflammatory properties [2, 3] and are used as perfumes and medicines [4]. The aim of this study was to identify antimicrobial compounds from the aerial parts of *C. atlantica*. The air-dried plant material (350 g) was macerated with *n*-hexane, CHCl₃, and CHCl₃-MeOH(9:1) to yield 8.26, 11.46, and 26.43 g of the respective residue. The chloroform extract at 125 µg/mL (MIC100) showed an interesting antimicrobial activity against *Acinetobacter baumannii*, a highly pathogenic, multidrug-resistant bacterium that have a significant threat to public health, making it a critical target for antimicrobial research.

The chloroform extract, the most active, was then separated by column chromatography on silica gel, yielding eight fractions, which were used to assess their effect on bacterial growth. Two fractions rich in abietane diterpenes affected bacterial growth by prolonging the lag phase. This suggests an effect on bacterial virulence as the bacteria require a longer adaptation time. The active fractions were therefore separated by RP-HPLC, yielding two new diterpenoids (1-2), two new megastigmane glycosides (3-4) (► Fig. 1) and several known abietanes, whose structures were elucidated by 1D and 2D NMR spectroscopy and HRMS experiments. The evaluation of the antibacterial activity of the isolates is currently under investigation.



► Fig. 1 Structure of compounds 1-4 isolated from *Cedrus atlantica* aerial parts

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P494 Microwave-assisted extraction of Triphala remedy for healthy gummy jelly production

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Triphala is a well-known Ayurvedic herbal formulation consisting of fruits from three plants: *Terminalia chebula*, *Terminalia bellerica*, and *Phyllanthus emblica*. This remedy not only aids in detoxification and digestion support but also provides a rich source of polyphenols and flavonoids that exert powerful antioxidant action [1]. Many studies have shown that regular consumption of Triphala can help promote the immune system, reduce inflammation, improve gut health, and even support weight management, making it a valuable addition to healthy products [2, 3]. This work aimed to develop an efficient microwave-assisted extraction (MAE) method with an edible sweet-tasting solvent, glycerin, to extract polyphenols from Triphala for making healthy gummy jelly. The effect of glycerin content (0–100 % w/w), solid-to-solvent mass ratio (1:5 to 1:20), power (200–500 watts), and duration (up to 4 cycles, 1 cycle = on 30 sec / off 30 sec) was investigated regarding the content of gallic acid and ellagic acid, which was analyzed using HPLC [4]. The optimum MAE condition was 25 % glycerin, 1:20 solid-to-solvent mass ratio, 200 watts, and 4 cycles, which yielded the maximum content of gallic acid and ellagic acid for 1,879.8 ± 45.5 and 765.8 ± 16.2 mg/g of extract, respectively. This extract has sweet and sour tastes. Our healthy gummy jelly contained gelatin, fructooligosaccharides, inulin, xylitol, honey, and flavoring agents, while the addition of Triphala extract resulted in a dark brown color. Overall, these findings suggested that the Triphala extract with this MAE condition might be useful for making functional gummy jelly for health-conscious consumers.

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P495 Quantifying allergens in *Rosa × damascena* hydrolats: Challenges of solvent-based extraction methods

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Hydrolats are increasingly recognized as valuable ingredients in modern cosmetic products, both as raw materials and as stand-alone formulations. Their natural origin and perceived gentleness contribute to their growing popularity

in skin care, particularly for sensitive and mature skin. However, despite their widespread use, there is a lack of standardized analytical approaches for verifying the presence and concentration of allergenic or restricted substances in hydrolats. These include compounds such as methyl eugenol, which are regulated under cosmetic legislation due to safety concerns [1, 2].

The aim of this study was to determine the composition and allergen content of rose hydrolats. Hydrolats were prepared by water distillation of *Rosa × damascena* flowers harvested at three different phenophases: early flowering, peak flowering, and late flowering. The samples were analyzed by GC-MS, both by direct injection and after extraction with hexane and ethyl acetate, to quantify total volatiles and allergens. The main compounds identified were phenethyl alcohol, geraniol, and nerol. Direct analysis revealed only these constituents, with phenethyl alcohol dominating (up to 70.32 % at peak flowering). Solvent extracts revealed a broader spectrum of volatiles, including allergens such as linalool, α -terpineol, citronellol, neral, and geranial. However, extraction-based methods significantly underestimated the concentrations of hydrophilic allergens. For example, geraniol reached 187 mg/L in hydrolats, compared to only 8.06 mg/L and 10.13 mg/L in hexane and ethyl acetate extracts, respectively. The analysis of commercial rose hydrolats confirmed the broader relevance of this discrepancy and underlined the need to adapt analytical methods to ensure accurate quantification of allergens for regulatory compliance and consumer safety.

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P496 A fern-based plant extract reduces the TNF- α -induced release of the pro-inflammatory cytokines IL-8, MIP-3 α , and IP-10 in immortalized human gastric MKN-45 cells

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Chronic inflammation of the gastric mucosa induced by the increased release of the *Helicobacter pylori*-associated pro-inflammatory cytokines interleukin-8 (IL-8), macrophage inflammatory protein-3 α (MIP-3 α /CCL20) and interferon- γ -induced protein 10 (IP-10/CXCL10) play a crucial role in the development of gastric diseases such as gastritis, peptic ulcer and gastric carcinoma [1–3]. A targeted reduction in the release of cytokines by plant-derived constituents, which has already been demonstrated in earlier studies with regard to pro-inflammatory interleukins [4, 5], could therefore be a promising approach for preventing tissue-damaging processes.

In this study, the effect of the commercially available fern-based extract Digestodoron® on TNF- α -induced release of IL-8, MIP-3 α and IP-10 in immortalized human gastric MKN-45 cells was assessed. Pretreatment of the cells with Digestodoron®, its four individual extracts (*Athyrium filix-mas*, *Phyllitis scolopendrium*, *Polypodium vulgare* and a mixture of *Salix alba*, *Salix viminalis* and *Salix vitellina*) or a commercial reference product, followed by a cytokine-inducing TNF- α incubation, led to a reduced release of IL-8, MIP-3 α and IP-10, which were quantified by ELISA. Dose response experiments showed that substantially lower concentrations of Digestodoron® (IC₅₀[IL-8] = 18 μ g/mL, IC₅₀[MIP-3 α] = 11 μ g/mL, and IC₅₀[IP-10] = 7 μ g/mL) were necessary to reduce cytokine release compared to the reference product investigated (IC₅₀[IL-8] = 412 μ g/

mL, IC₅₀[MIP-3α] = 160 µg/mL, and IC₅₀[IP-10] = 45 µg/mL). The results indicate an anti-inflammatory potential of the fern-based plant extract in gastric diseases, which has to be validated *in vivo*.

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P497 Pre-formulation test: Impact of *Thymus serpyllum* L botanical extract (360GUT®) on probiotic viability

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A proprietary ingredient developed from the aerial parts of *Thymus serpyllum* L called 360GUT® has significantly improved gastrointestinal symptoms and positively modulated the microbiota in healthy volunteers [1]. Its benefits have been attributed to the presence of a specific polyphenol profile, which are prebiotics that feed the beneficial gut microbiome. Otherwise, probiotic drinks such as yoghurts and fermented milk are widely consumed, as probiotics are live microorganisms that also confer health benefits to the host when administered in adequate amounts. Prebiotics and probiotics can be combined for optimal effect, but it is essential for any product to ensure that the probiotic strains remain viable and metabolically active during product storage. The aim of this study was to assess whether the probiotic strains remain unaffected in the presence of 360GUT®. Two commercial probiotic drinks, containing *Lactobacillus casei* Shirota or *Bifidobacterium lactis*, were separately dosed with 600 mg of 360GUT® per serving and stored at 4–8 °C for up to 14 days. Culturability and viability were assessed by plate counting and flow cytometry, respectively, and statistical differences were considered with a 95 % confidence interval ($p < 0.05$) using a Student's *t*-test [2]. The results show that the population density of both bacteria was unaffected by the presence of 360GUT® at the days 0, 7 or 14 in the both methods, demonstrating that the probiotics are still viable and metabolically active. It was concluded that 360GUT® is a highly versatile ingredient that can be formulated in various applications, including dairy drinks.

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P498 Formulation and evaluation of microemulsion containing a Thai traditional remedy extract for treatment of dermatitis

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Dermatitis is a skin inflammatory disease associated with epidermal barrier dysfunction, often causing redness and pruritus [1]. In Thai traditional medicine, the polyherbal remedy called “Ruean-Mhoon-Nok (RMN)” has been utilized as a topical treatment for dermatitis and psoriasis. This remedy composed of 9 plants, such as *Piper wallichii*, *Alpinia galanga* and *Datura metal*. Our recent study revealed antioxidant ability of RMN ethanolic extract with high phenolic and flavonoid contents [2]. The extract and its bioactive compound, hydroxy-chavicol, also demonstrated to manage inflammation by inhibiting NO and IL-6 productions [3]. This study focused on developing a stable microemulsion for skin delivery of RMN extract using various oil, surfactants, and co-surfactants. The optimal formulation was selected from a pseudo-ternary diagram with large microemulsion region, which consisted of propylene glycol mono-caprylate (5 %w/w) with a mixture of PEG-8 caprylic/capric glycerides and ethoxydiglycol (50 %w/w at 1:2 ratio), and RMN extract (1 %w/w). This formulation displayed small droplet size (42.8 ± 0.4 nm, PDI of 0.182 ± 0.005) with negative charge (-54.56 ± 1.61 mV), low viscosity (13.53 ± 0.23 cP) and slightly acidic pH (4.47 ± 0.01). In vitro permeation experiment using Franz diffusion cells indicated that the microemulsion could penetrate through the Strat-M® membrane and release 26.8 ± 0.3 % of hydroxychavicol over 8 hours. In addition, it was stable after exposed to extreme temperature change (heating-cooling for 9 cycles). Overall, this RMN microemulsion could be useful for the clinical application to treat dermatitis.

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P499 The peppermint oil contained in Digestopret® acts anti-inflammatory, anti-spasmodic and modulates human microbiome and its function

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According to the German S3 guideline for irritable bowel syndrome (IBS) [1], peppermint oil, as contained in Digestopret®, can be used for symptomatic relief of minor spasms of the gastrointestinal tract, flatulence and abdominal pain, especially in patients with IBS.

Here we confirm its well-known antispasmodic and anti-inflammatory effects and show that peppermint oil acts directly on the intestinal human microbiome to reduce gas production. To investigate the anti-inflammatory potential of peppermint oil, RAW 264.7 macrophages were seeded in 24-wellplates and stimulated with 100 ng/ml LPS. TNF secretion was measured by ELISA. Spas-

molytic activity was assessed by testing peppermint oil on colon biopsies from five male guinea pigs. Effects on the microbiome and its metabolism were assessed using an anaerobic *in vitro* fermentation system with stool samples from six healthy donors by quantitative 16s rRNA gene sequencing and measurement of selected activity parameters, such as gas production.

Peppermint oil showed a dose-dependent reduction of LPS-induced TNF secretion from RAW 264.7 macrophages and concentration-dependent anti-spasmodic effects on colon biopsies. Moreover, peppermint oil significantly reduced microbial gas production, potentially alleviating bloating, while alpha diversity indices were not affected. At the genus level, the growth of potential pathogens such as the genus *Escherichia-Shigella* or *Parabacteroides* was reduced, while the growth of health-related organisms (e.g. *Blautia*, *Faecalibacterium*) was promoted. The results obtained show that besides antispasmodic and anti-inflammatory effects, peppermint oil exhibits direct and indirect effects on the human gut microbiome as well as gas production contributing to successful IBS treatment with Digestopret®.

Conflicts of interest All authors are employees of Bionorica SE, the manufacturer of Digestopret®.

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P500 Potential of *Salicis cortex* as a treatment option for inflammatory bowel disease

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Salicylic acid derivate 5-ASA is still a standard for maintenance therapy of Ulcerative Colitis (UC) but often with limited efficacy and adverse effects when used chronically. Therefore, extracts from *Salicis spp.* containing derivatives of salicylic acid may be a safe and effective alternative. Extracts of *Salix spp.*, are indicated for reducing lower back pain or minor articular pain due to their anti-inflammatory properties according to EMA monograph [1]. Thus, the potential of a *Salix purpurea* extract for the treatment of UC was investigated *in vitro* and *in vivo*. Anti-inflammatory effects were analysed using PBMCs from patients suffering from UC or Crohn's disease, as well as from healthy donors. After stimulation with LPS, secretion of PGE2 or various cytokines was measured. A DSS-induced colitis model in mice was used to assess anti-inflammatory effects *in vivo*. To explore the potential role of the gut microbiome, effects on the microbiome were studied in a specific anaerobic fermentation system using Next-Generation-Sequencing. The extract reduced the secretion of some inflammatory markers from PBMCs of healthy and diseased donors in a concentration-dependent manner. In addition, a dose-dependent amelioration of DSS-induced inflammation was observed in mice. Moreover, microbiome analysis revealed the growth of health-related organisms (e.g. *Catenibacterium* or *Blautia*), was promoted, while alpha diversity indices remained unchanged. The investigated *Salix* extract showed anti-inflammatory effects *in vitro* and *in vivo* and modulating effects on the gut microbiome, exhibiting potential as maintenance treatment after induction of remission by immunosuppressives and/or biologics following confirmation of efficacy in clinical trials.

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P501 Rare natural fatty acids: unlocking the potential of petroselinic acid as anti-obesity and antiinflammatory agent

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Rare natural fatty acids are frequently found in oil-rich seeds of specific plant families, and they are usually distinguished for their biological potential. Within this class, petroselinic acid (PA) is a monounsaturated fatty acid predominant in the seeds (schizocarps) of Apiaceae and has gained notable interest for the numerous biological properties. Among these, its inhibition of the bacterial lipase has been reported. However, its potential as anti-obesity agent still needs in-depth investigation [1]. In this study, 11 Apiaceae seeds were screened for their PA content, and an innovative UHPLC-MS method was developed for its quantification. Notably, aniseed was found as the richest source of PA (3.97 %). Furthermore, a purification procedure was set up, leading to a high purity of PA devoid of its positional isomer oleic acid (recovery of 45 %). Thus, the anti-inflammatory and anti-obesity properties of PA were assessed in terms of inhibition of lipoxygenase (LOX) and phospholipase A2 (PLA2), and porcine pancreatic lipase (PPL), respectively. The results revealed a strong inhibition of PPL, with a 90–85 % activity reduction at 1000 µg/mL, indicating a comparable efficacy to that of the anti-obesity drug orlistat. Additionally, the inhibition of LOX demonstrated a significant anti-inflammatory effect, with an 85–75 % activity reduction at 1000 µg/mL. The prevention of lipid accumulation was evaluated on *Caenorhabditis elegans*, a widely used *in vivo* model for the screening and identification of anti-obesity compounds [2]. This study demonstrated the considerable therapeutic potential of PA as anti-obesity and anti-inflammatory agent encompassed with favorable safety profile.

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P502 Effects of *Sideritis scardica* Griseb extract (Greek mountain tea) on anxiety in menopausal and postmenopausal women

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Menopause is a transitional phase characterized by significant hormonal fluctuations, which can negatively impact the nervous system, often leading to increased anxiety. Women are particularly vulnerable to anxiety during this period. *Sideritis scardica* has demonstrated potential anxiolytic effects in previous studies [1], as well as a standardized hydroethanolic extract, Concentral®. A sub-analysis was conducted using data from a clinical study performed at the Brain Performance and Nutrition Research Centre, Northumbria University [2]. From the total dataset (n = 142), a subgroup of 85 women aged 50 and above was analyzed to assess the effects of *Sideritis scardica* extract on anxiety. Women aged 50–59 were considered menopausal, and those over 60 postmenopausal.

sal. The intervention consisted of daily supplementation with either 475 mg or 950 mg of Concentral® for 28 days. Anxiety was assessed using state and trait anxiety scales. Compared to placebo, both doses of Concentral® significantly reduced state anxiety after 28 days in the total female subgroup. Specifically, 475 mg showed the greatest improvement in women aged 50–59, while 950 mg was more effective in postmenopausal women. Furthermore, 950 mg Concentral® also significantly reduced trait anxiety in women over 60, indicating a longer-term benefit. Concentral® appears to be an effective natural option for reducing anxiety in menopausal and postmenopausal women. The results suggest a dose- and age-dependent response, with 475 mg being more effective during menopause and 950 mg offering benefits in postmenopause. These findings support the use of Concentral® as a targeted intervention for managing anxiety symptoms in this population.

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P503 Efficacy and safety of CLSi017 in managing menopausal symptoms: A randomized, double-blind, placebo-controlled clinical trial of *Asparagus racemosus* root extract

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Menopause is a significant transitional phase in women's lives characterized by hormonal fluctuations that often involve uncomfortable symptoms affecting quality of life. Though hormone replacement therapy may alleviate some issues, it is also associated with increased risk of cardiac events and some cancers [1]. Shatavari (*Asparagus racemosus*) has been long utilized in traditional Ayurvedic medicine to support women's reproductive health, though rigorous clinical evidence for its efficacy remains limited [2].

In this study, we evaluated standardized *A. racemosus* root extract (CLSi017) in improving menopausal symptoms across somatic, psychological, and urogenital domains. 60 healthy (pre-, peri-, and post-menopausal) women aged 40–55 years were recruited and randomized into 2 groups to receive daily doses of either 100 mg of placebo-brown rice starch or CLSi017-5 % Shatavarin IV root extract (double-blinded administration). Menopausal symptoms were assessed at baseline (pre-treatment) and at 4- and 8-weeks (post-treatment) using the Menopause Rating Scale (MRS). Confirming successful randomization, both groups reported similar baseline MRS scores (~15 on a 44 point scale). However, post-treatment, the CLSi017 group demonstrated significant reduction in menopausal symptom severity at week 4 (43 % decrease) and at week 8 (71 % decrease, linear mixed model LMM analysis, $p < 0.001$). Additionally, various parameters across MRS subscales showed significant improvement including hot flash occurrence and sleep quality (both >90 % improved at week 8). Adverse events were mild, transient, and comparable between groups, with gastrointestinal discomfort being the most common.

These findings suggest that CLSi017 may represent a promising botanical alternative for women seeking non-hormonal approaches to manage menopausal symptoms.

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P504 The acute metabolic impacts of *Kaempferia parviflora* extract in healthy men: A randomized, double-blind, proof-of-concept study

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Obesity and related comorbidities represent a growing global health concern. This study assessed the effects of GyngerLean™, a supplement containing *Zingiber officinale* (yellow ginger), *Alpinia galanga* (red ginger), and *Kaempferia parviflora* (black ginger), on energy expenditure and substrate utilization. Ten healthy adult males participated in this randomized, double-blind, two-dose crossover trial. Participants received either 100 mg or 200 mg doses of GyngerLean™ with a minimum 24-hour washout period between visits. Resting energy expenditure (REE) and respiratory exchange ratio (RER) were measured at baseline and at 60-, 120-, and 180-minutes post-supplementation using indirect calorimetry. No significant differences were observed at baseline between the 100 mg and 200 mg doses for REE (100 mg: 2203 ± 497 kcal vs. 200 mg: 2454 ± 501 kcal; $p = 0.2408$) or RER (100 mg: 0.79 ± 0.09 vs. 200 mg: 0.81 ± 0.04 ; $p = 0.4911$). The 100 mg dose showed no significant changes in REE or RER over time. For the 200 mg dose, REE remained stable across all timepoints, but RER decreased significantly at 120 minutes (0.77 ± 0.05 , $p = 0.0158$) and 180 minutes (0.75 ± 0.04 , $p = 0.0004$) post-consumption compared to baseline, indicating increased fat oxidation relative to carbohydrate oxidation. Acute ingestion of 200 mg GyngerLean™ significantly enhanced fat oxidation in healthy men without affecting overall energy expenditure. These findings suggest the potential role of ginger extracts in promoting metabolic changes that could support weight and body composition management [1, 2]. Future research should investigate whether chronic supplementation would affect weight and body composition and explore the underlying mechanisms through which ginger influences substrate utilization.

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P505 The impact of herb extraction techniques on phytochemicals content and their efficacy in the prevention of gastrointestinal diseases

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Digestive system diseases are a significant health and economic problem worldwide. The long and expensive nature of their treatment leads people to look for preventative solutions and natural remedies. Plant-derived bioactive compounds (phytochemicals) deliver multiple health-promoting effects, and their appropriate use can effectively support the prevention and treatment of digestive system diseases. The study aimed to compare various extraction methods commonly available to consumers (infusions, two types of decoctions and macerates) and to optimise their parameters (temperature and duration). Based on literature data, 10 plant species used to support the treatment of digestive system diseases were selected. The content of polyphenols in the obtained extracts – some of the most active phytochemicals – was determined, and their antioxidant potential was assessed using ABTS, FRAP and FeMICA tests. The results showed that 30-minute decoctions were the most effective extraction method for most of the analysed plants due to the highest polyphenol

nol content and antioxidant potential, expressing the ability to eliminate free radicals (ABTS), as well as reducing and chelating properties (FRAP and FeMICA, respectively). Infusions, preferred by consumers, were found to be the least effective. These results clearly showed that the choice of the proper extraction method is crucial for the efficiency of extracting valuable phytochemicals and, as a result, the effectiveness of phytotherapy. The optimisation of the extraction process can increase the bioavailability and efficacy of plant preparations, which, together with proper diet and lifestyle, can be a critically important element of prevention and supportive therapy.

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P506 Schisandrin C ameliorates indomethacin-induced intestinal injury: An integration study of network pharmacology, *in vitro* cells, and *in vivo* *Caenorhabditis elegans*

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The long-term administration of indomethacin, a nonsteroidal anti-inflammatory drug (NSAID), has been associated with severe gastrointestinal side effects by inducing inflammation, oxidative stress, and downregulating tight junction protein expression [1, 2]. *Schisandra chinensis* (Turcz.) Baill., an herbal medicine for treating gastrointestinal disorders, can potentially ameliorate these adverse effects [3]. This study aimed to discover the bioactive compound from *S. chinensis* and elucidate its mechanism of action underlying the protection against indomethacin. Network pharmacology analysis indicated that schisandrin C (SCHC), which exhibits the best drug-like properties, is the active ingredient of *S. chinensis* to protect against indomethacin-induced intestinal injury. The analysis revealed that SCHC might exhibit protection through various targets, such as AKT1, PIK3CA, GSK3B, RELA, and CASP9. Molecular docking and molecular dynamics simulation data indicated that SCHC has high binding affinities with GSK3B and PIK3CA. *In vitro* Caco-2 cell experiments indicated that SCHC reduced apoptotic cell death and intestinal inflammation and upregulated tight junction protein expression by modulating the AKT/GSK-3 β signaling pathway. In addition, SCHC could effectively reduce intestinal atrophy and increase the intestinal lysosomal level in *Caenorhabditis elegans* induced by indomethacin. The protective effect of SCHC in *C. elegans* was also demonstrated to be associated with the PI3K/AKT signaling pathway through the loss in bioactivity of SCHC in mutant strains of *age-1*, *akt-1*, and *akt-2* genes. In conclusion, by integrating computational predictions and experimental validation, this study suggests that SCHC can be a promising candidate for protecting against indomethacin-induced intestinal injury.

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P507 Phytotherapy of mood disorders: studies on the role of probiotics in biotransformation of plant compounds

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Herbal extracts have long been used to manage mood disorders, with many showing strong clinical evidence for their effectiveness. Nevertheless, the mechanisms of their action and the active compounds remain unknown [1]. Recent research highlights the crucial role of gut microbiota in the biotransformation of plant compounds into new bioactive metabolites, known as postbiotics [2–4]. However, there is still a lack of research on the role of probiotics in the biotransformation of plant compounds and their impact on the phytotherapy.

This study investigates the biotransformation of plant compounds of four herbal extracts – *Valeriana officinalis* L. root, *Passiflora incarnata* L. herb, *Piper methysticum* G. Forst. rhizome, and *Humulus lupulus* L. female inflorescences – by selected, commercially available probiotics. Phytochemical analysis confirmed the presence of characteristic plant compounds, including iridoid glycosides, valepotriates, flavonoids, caffeic acid derivatives, kavalactones, flavokavains and bitter acids, in the extracts. Biotransformation studies indicated potential role of probiotics in the metabolism of plant compounds. *Lactocaseibacillus rhamnosus* strain was responsible for the formation of two postbiotics, C-glycoside derivatives of apigenin and luteolin, from compounds of *Passiflora incarnata* extract. Additionally, *Lactobacillus helveticus*, *Bifidobacterium longum* and *Saccharomyces boulardii* strains, were implicated in the metabolism of iridoid glycosides and bitter acids. However, major kavalactones, including kavain, 7,8-dihydrokavain, desmethoxyyangonin, yangonin, and dihydromethysticin, remained unaltered by all probiotic strains.

These findings suggest that probiotics may influence the bioavailability and pharmacological activity of compounds of mood-modulating herbal extracts. Further studies into combined herbal-probiotic therapies could offer a promising approach for treatment of mood disorders.

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P508 Alcoholic fermentation of different *Vaccinium* species: optimization and phytochemical analysis of berry wines

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Edible berries are an important part of nutrition, they are often preserved as jams, jellies and beverages. Alcoholic fermentation is one of the commonly used methods of fruit preservation, but not all berry species are suitable. In this study, three closely related blueberry/bilberry species were tested for their suitability and quality of the resulting berry wine, namely *Vaccinium myrtillus*,

V. uliginosum and *V. corymbosum*. In the first step, the three species were fermented either mashed or juiced (► Fig. 1). The best result was achieved with *V. uliginosum*; *V. myrtillus* fermentation failed due to mold and *V. corymbosum* wine, despite the higher content of alcohol in the final product, showed a significantly lower content of health-promoting secondary metabolites and less pleasant aroma. In the second step, juice of *V. uliginosum* was optimized for alcoholic fermentation by the addition of sugar and/or yeast. Higher alcohol content and higher pH was achieved in the sweetened juices, both with natural yeast and inoculated *S. cerevisiae*. The addition of yeast resulted in significantly earlier onset of fermentation and a higher phenolic content in the product. Commercial yeast strain also partially attenuated the anthocyanin loss during the fermentation process. Compared to the other related species of blueberry, the bog bilberry *V. uliginosum* appears to be the most suitable for the preparation of alcoholic beverages. This particular species is still underutilized for food and nutritional supplement production; therefore, this study highlights a novel natural resource for this purpose.



► Fig. 1 The experimental setup for the berry juice fermentation

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P509 3D-Printed composite scaffolds coated with collagen and tetracycline for enhanced bone regeneration and antibacterial activity

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Bones play a crucial role in the human body by providing mechanical support, enabling movement, and protecting vital organs. Although bone is a vascularized tissue capable of self-regeneration, large defects, severe trauma, or conditions such as infections or metabolic disorders, often require surgical intervention. Treatments typically involve autografts, allografts, or xenografts, each presenting limitations like donor site morbidity, limited availability, immune rejection, and the risk of disease transmission [1]. Tissue engineering presents a promising solution. Advances in 3D bioprinting and biomaterials allow the development of bone scaffolds specifically designed for regeneration. These structures can also serve as drug delivery systems (DDS) by incorporating therapeutic agents, such as antimicrobial or anti-inflammatory compounds, directly into the scaffold. The inclusion of antimicrobials is particularly important to minimize the risk of infection during and after implantation [2]. In this study, 3D-printed composite scaffolds were coated with a collagen type I solution containing the broad-spectrum antibiotic tetracycline hydrochloride (TCH). The scaffolds consisted of poly(ϵ -caprolactone) (PCL) and β -tricalcium phosphate (β -TCP) at a 70:30 w/w ratio and were fabricated using the BIO X Bioprinter (Cellink, Sweden). Collagen type I was reconstituted in 0.02 M acetic acid at 500 μ g/mL. A 0.25 M TCH stock solution was diluted to final concentrations of 2.5, 5, and 10 mM. Scaffolds were immersed in these solutions, incubated at 37 °C with 5% CO₂ for 2 hours, and airdried for 6 hours. The drug-loaded scaffolds demonstrated antibacterial activity against *Staphylococcus aureus* and satisfactory biocompatibility, supporting their potential in bone tissue engineering applications.

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P510 Investigation of natural products from medicinal cannabis as SARS-CoV-2 infectivity inhibitors

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The *Cannabis sativa* plant has been utilized across various cultures for diverse applications, including as a source of food, fiber, and medicinal compounds. Historical records suggest that the cultivation of *Cannabis* for medicinal use can be traced to approximately 2700 years ago [1]. The plant's therapeutic properties, including antiviral activity, have been increasingly recognized, with cannabinoids and terpenes commonly identified as the primary bioactive constituents responsible for these effects [2].

In the present study, three distinct chemovars of *Cannabis sativa* were analyzed. A total of fourteen cannabinoids and thirteen terpenoids were identified using high-performance liquid chromatography with diode-array detection (HPLC-DAD), gas chromatography with flame ionization detection (GC-FID), and gas chromatography-mass spectrometry (GC-MS). Crude extracts from one of the chemovars were subjected to flash liquid chromatography (flash-LC) to obtain fractions. These crude extracts, their corresponding fractions, and isolated standard compounds were subsequently evaluated for antiviral activity using a cytopathic effect (CPE)-based bioassay with SARS-CoV-2 as the viral agent. The antiviral assessment indicated a potential correlation between the concentration of cannabinoids and terpenes and the observed antiviral efficacy. Notably, the full-spectrum extracts exhibited a higher antiviral effect compared to the fractions, while the isolated cannabinoids demonstrated the lowest activity. Among the tested cannabinoids, Δ^9 -tetrahydrocannabinolic acid (Δ^8 -THCA) showed higher antiviral potency than cannabidiol (CBD), with Δ^9 -tetrahydrocannabinol (Δ^8 -THC) exhibiting the lowest activity. These findings suggest that the antiviral properties of *Cannabis sativa* may be attributed to a

synergistic interaction between multiple phytochemicals, rather than the effects of individual compounds alone.

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P511 BIOCHRYSUM Project: the short supply chain of organic *Helichrysum* spp. to produce bioactive phytochemicals

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Helichrysum spp. are widely cultivated in the Tuscan Archipelago for the formulation of perfumes and other cosmetic preparations, thanks to the recognized anti-inflammatory, antibacterial and antiviral properties of this plant [1]. The BIOCHRYSUM project, funded by the Ministry of Agriculture, Food Sovereignty and Forests (MASAF) for research in organic farming (call no. 9220340, 2020), aims to develop a sustainable production of *Helichrysum* spp essential oil and extracts starting from genetically characterized native populations. The aims are to valorise the by-products after distillation (hydrolats and exhausted biomass) and to optimise the production of extracts for cosmetic, food and health applications. Alternative uses of plant extracts as biostimulants and biopesticides, as well as for the containment of biotic and abiotic stresses, will be tested. In order to optimize the stability, the biopharmaceutical and technological characteristics of the constituents, both conventional and innovative formulations (nanocarriers) will be developed. A key objective of the project will be the creation of a blockchain-based traceability system to monitor post-harvest phases through a database managed by all partners in the supply chain. The project is aligned with the Consolidated Law on Medicinal Plants (Legislative Decree 75/2018), which recognizes post-harvest operations, including distillation, as activities specific to the agricultural company. Partners are CREA-OF, the University of Florence (DAGRI, CHIM and CIBIACI), and some agricultural companies: Essenziale (FI), Le Giunchiglie (GR), La Piana (LI), Arura (LI), San Rocco (LI). Partners in kind: Agricultural Technical Institute – ITAGR (FI), Tuscan Archipelago National Park Authority, Municipality of Capraia Isola (LI), SEMIA agricultural company (FI).

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P512 Environmentally friendly microemulsions of curcumin and mangiferin to protect crops against *Fusarium verticillioides*

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The significant increase in the use of chemical pesticides to combat crop diseases has created a growing awareness among many consumers because of the side effects of these pesticides on human health and the environment [1]. Indeed, pesticide residues have become the focus of attention of European bodies responsible for food safety. In May 2020, the European Commission developed directives linked to the reduction of the use of pesticides with two goals: decrease the use and risk of chemical pesticides by 50 % by 2030 and reduce the use of the most dangerous pesticides by 50 % by 2030 [2]. The search for alternative solutions to protect crops has become urgent and has recently attracted widespread attention from researchers around the world focusing on natural products which represent a treasure chest of molecules with potent activities. Among polyphenols, curcumin reported to be active against *Fusarium* species [3], while mangiferin has shown activity against postharvest fungal pathogens [4]. Due to the low water solubility of both polyphenols, microemulsions which are homogeneous thermodynamically stable transparent dispersions were selected as nanovectors. The selected microemulsions were developed using vitamin E acetate as oil and Cremophor RH 40 and Labrasol ALF as surfactants by using a pseudo-ternary diagram, loaded with 10 mg/mL of polyphenols and evaluated against *Fusarium verticillioides* strains. Activity against *F. verticillioides* was tested using amphotericin B as the positive control. *F. verticillioides* was very susceptible to both nanoformulations. Both polyphenols remained very active at a dose of 5 mg/mL, with a four log reduction of *F. verticillioides*.

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P513 Improved antibacterial potential of *Cistus salvifolius* aerial parts on skin pathogens by optimization of cold and hot water extraction

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Cistus salvifolius is one of the most spread *Cistus* species in Cyprus and is a plant rich in phenolic compounds, associated with potent antimicrobial effects [1]. There is accumulated knowledge for the inhibitory effects of *Cistus salvifolius* on clinical and foodborne bacteria [2]. Previous studies also reported the antimicrobial potential of *Cistus* plants on skin pathogens such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* [3]. The aim of the present work was to optimize cold and hot water extraction of antimicrobial substances from *Cistus*

salvifolius using response surface methodology improving the antimicrobial potency against two common skin pathogens. In particular, the effects of extraction temperature, extraction time and water to plant material ratio on total phenolics, total flavonoids, total tannins and the inhibition of growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* expressed as optical density were studied using Box-Behnken designs. According to the desirability index, the highest antimicrobial potency could be obtained by extraction plant material at 7 °C or 88 °C for 300 or 13 min by mixing water and plant material at a ratio of 125 mL per g for cold and hot water extraction, respectively. Subsequently, minimum inhibitory and minimum bactericidal concentrations as well as drug-enhancing and biofilm formation inhibition activity were also determined for both extracts. Overall, the present study demonstrates the potential of aqueous extracts of *Cistus salvifolius* to control the growth of skin pathogens and delivers guidelines for the preparation of cold and hot and cold water extracts.

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P514 Quality, purity and labelling accuracy of marketed herbal products: the case of *Andrographis paniculata*

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Current herbal food supplement regulations do not systematically impose quality assessments prior to commercialisation, leaving the quality of these products largely unknown [1]. With increasing popularity of *Andrographis paniculata* supplements since the COVID-19 pandemic and their easy consumer access from pharmacies as well as online, understanding the quality of these products is crucial [2]. This study aims to assess the composition, purity, and labelling accuracy of *A. paniculata*-containing products.

In this quality control study of 40 herbal products containing *A. paniculata*, 13 were purchased from pharmacies and 27 online. All samples except one contained *A. paniculata*. The daily dose of andrographolide of the 33 solid herbal products was comparable using UHPLC-UV and UHPLC-MS, except for two products containing quercetin which interfered with the analysis. Of the 23 products for which the labelling contained the dose of andrographolide, measured daily dose of andrographolide ranged from 29 % to 174 % of the labelled dose; two products were within the 10 % tolerance range, 20 lower and one higher.

This study reveals widespread mislabelling and underdosing in *A. paniculata*-containing herbal food supplements marketed internationally. This highlights the need for relevant regulatory authorities to enforce quality controls of HFS to ensure safety and transparency for the consumer.

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P515 Fruitful Prospects: investigating the metabolic benefits of *Chaenomeles japonica*, *Cornus mas*, and *Hippophaë rhamnoides* extracts in *Caenorhabditis elegans*

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Secondary metabolites of *Chaenomeles japonica* (CJ), *Cornus mas* (CM), and *Hippophaë rhamnoides* (HR) fruits have been proposed as hypoglycemic and hypolipidemic agents *in vitro* [1]. To further investigate their health-promoting potential, this study evaluated the effects of standardized aqueous (CJ, HR) and 70 % hydroethanolic (CM) extracts on survival (N2 wild-type), fat accumulation (SS104), and β -amyloid-induced proteotoxicity (CL4176) in the *Caenorhabditis elegans* model [2]. The presence of isorhamnetin glycosides, procyanidins B1 and B2, epicatechin, loganic acid, and cornuside in the respective extracts was confirmed using ultra-high-performance liquid chromatography coupled with triple quadrupole mass spectrometry (UHPLC-TQMS, Xevo TQ Absolute). Previously, only HR-derived products or total HR flavonoid fractions have been reported to affect hyperglycemia and delay paralysis in *C. elegans* [3, 4].

Survival assays demonstrated good extract tolerability up to 200 μ g/mL. Both CJ and CM slightly improved worm survival across all tested concentrations, with 200 μ g/mL CM significantly enhancing survival by 23 % compared to the vehicle control. CM at this concentration also significantly increased fat accumulation, while HR showed a slight fat-reducing effect. None of the extracts influenced β -amyloid toxicity in the CL4176 strain.

These findings indicate that the tested extracts may possess greater therapeutic potential in the context of metabolic disorders than for neuroprotective conditions, in contrast to previous *in vitro* indications.

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P516 Fermented kombucha tea: A dual source of bioactive compounds from tea and Symbiotic culture of Bacteria and Yeasts (SCOBY)

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Kombucha is a fermented tea beverage recognized for its antioxidant, antimicrobial, and antifungal properties, offering potential health benefits [1]. This study investigates the preparation of kombucha using high-quality green tea. To prepare the tea, 5 g of tea leaves are added to 1 L of boiling tap water and infused at temperatures between 70 and 95 °C for no more than 15 minutes. Organic, unrefined sugar serves as the fermentation substrate. The SCOBY, a consortium of bacteria and yeast, is sourced from Medica Laboratories in Romania and cultivated in a sugar-based medium containing a 3% (w/v) green tea infusion to promote the microbial activity necessary for fermentation. The fermentation process begins by adding the SCOBY and sugar to the cooled tea mixture. Primary fermentation occurs for 10–14 days at room temperature (22 ± 2 °C), with ultrasonication applied to break down plant cells and release nutrients, stimulating microbial activity. Aeration introduces sterile oxygen to enhance yeast growth, while artificial lighting influences the production of secondary metabolites. The kombucha is then transferred to airtight glass bottles for maturation over 1–2 weeks to achieve natural carbonation (secondary fermentation). Metabolic profiling using UHPLC-Q-Orbitrap-HRMS reveals bioactive metabolites. The kombucha demonstrates antioxidant activity (2.331–2.506 µM TE/mL) and significant antimicrobial and antifungal activity against *E. coli*, *S. aureus*, *B. subtilis*, and various fungal pathogens. These findings highlight the functional potential of SCOBY-based kombucha as a beverage with remarkable bioactive properties.

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P517 Real-World assessment of effectiveness and tolerability in utilization of herbal medicinal products for gynecological complaints with regard to Co-Medication

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Little is known about the effectiveness and tolerability of herbal medicinal products (HMPs) when they are utilized concomitantly with synthetic drugs. Therefore a sample (n = 1658) was taken from the pharmaco-epidemiological database PhytoVIS consisting of real-world outcomes reported by women treating their gynecological complaints with HMPs [1]. We focused on *Vitex agnus-castus* L. (VAC) (n = 197) or *Actaea racemosa* L. (CR) (n = 223) because their therapeutical benefits and safety have already been clinically proven [2, 3]. The vast majority of women using only VAC (n = 177) or only CR (n = 113), experienced a good effectiveness (~92 %) and only a minor share did not benefit (~9%). When VAC was taken alongside other drugs the effectiveness was still high (91 %, n = 78), and only a few (9 %, n = 7) did not perceive any symptom relief. In these cases adverse events did not occur. Similar results have been observed with CR. When it was utilized concomitantly with other drugs (n = 110), most women (96.4 %, n = 106) perceived the effectiveness as good. Only a few (3.6 %; n = 4) complained about undesired events. Interestingly, the number of combined medications did not affect the perceived effectiveness (p = 0.119; r = -0.114) or tolerability (p = 0.279; r = -0.079) of VAC or CR. Our results show that the perceived effectiveness and tolerability of CR and VAC is independent of whether they are taken as monotherapy or combination therapy.

In addition to comedication, other factors e.g. daily dose, duration & frequency of use or concomitant diseases should be investigated, as these could also have an influence on the perceived effectiveness and tolerability.

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P518 Chemical analysis of the polyphenolic fraction in industrial Hemp vr. *Kompolti* infusions: metal analysis, antioxidant capacity, and cannabidiol in vitro neuroprotective qualities

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The effects of the main component, cannabidiol (CBD), were rationalized at the molecular level in a Parkinson's disease (PD) model through a multidisciplinary approach that included NMR, MS, in vitro cell-free and cell-based assays, and in *silico* studies [1]. The phytochemical analysis performed by LC-MS/MS, led to the tentative identification of numerous components from various polyphenol classes, including phenolic acids, flavonoids, and their glycosides. Along with trace amounts of other cannabinoids, the infusion also contained significant levels of CBD and CBDA. Additionally, the water infusion (WI) was assessed for mineral content, total phenolic content, flavonoid concentration, and antioxidant capacity using the DPPH and FRAP methods. Our findings in a PD cellular model are noteworthy, showing that CBD prevents rotenone-induced cell death without restoring neuronal architecture. An in *silico* method was used to rationalize these biological results, and we hypothesize that CBD affects the cellular response to oxidative stress through its interaction with the Keap1/Nrf2 pathway. In conclusion, these findings enhance the nutraceutical profile of the water infusion from the *Kompolti* cultivar's inflorescences, which

showed a high concentration of CBD. This research could lead to the development of dietary supplements aimed at alleviating clinical symptoms associated with CBD's antioxidant activity in the poorly understood pathophysiology of Parkinson's disease.

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P519 Protective effects induced by a novel formulation based on resveratrol and water extracts from *Equisetum arvense*, *Crataegus curvisepala*, *Vitex agnus-castus*, and *Glycine max* in C2C12 cells exposed to oxidative stress

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Medicinal plants and natural compounds have been considered alternative therapeutic options for treating symptoms of postmenopausal disorders thanks to their different concomitant effects, including antioxidant and anti-inflammatory properties and the regulation of hormone activity. The efficacy of medicinal plants and natural compounds may increase when used in combination. Therefore, the present study evaluated the phytochemical and pharmacological properties of an innovative formulation consisting of resveratrol and water extracts of *Equisetum arvense*, *Crataegus curvisepala*, *Vitex agnus-castus*, and *Glycine max* [1]. The phenolic composition and radical scavenging properties were evaluated using chromatographic and colorimetric (ABTS) methods, whilst the limits of biocompatibility were assessed through allelopathy, the *Artemia salina* lethality test, and *Daphnia magna* cardiotoxicity assay. Protective effects were evaluated on C2C12 cell lines exposed to hydrogen peroxide. The gene expression of estrogen 1 (ESR1) and prolactin (PRLR) receptors, interleukin 6 (IL-6), tumor necrosis factor α (TNF α), and receptor activator of nuclear factor kappa-B ligand (RANKL) was measured. The results of the phytochemical analysis showed that the main phytochemicals were hydroxycinnamic and phenolic acids. This could explain the radical scavenging effect observed in the ABTS assay. In the proposed ecotoxicological models, the formulation was revealed to be non-toxic, with a LC50 value > 1 mg/mL. In C2C12 cells the formulation (200–1000 μ g/mL) decreased the hydrogen peroxide-induced upregulation of TNF α , IL-6, RANKL, ESR1, and PRLR. Overall, the results of this study corroborate the use of the formulation for facing oxidative stress and inflammation, which forms the basis of the osteoclastogenic process.

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P520 Estragol: How much is too much?

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There are different approaches to assess the safety of trace constituents of natural products. Given the recent regulatory awareness of estragol, a widely spread constituent of medicinal and food plants [1], results from such approaches could have direct regulatory impact.

To leverage recently generated such data, relevant in vitro and in vivo studies have been retrieved, presented and assessed [2].

New data support this view. The number of DNA adducts in human hepatic samples was significantly below numbers correlated to mutagenicity in HepG2-CYP1A2 cells in vitro [3–5].

The scientific evidence for a threshold of mutagenic effects of estragole above exposures occurring with the intake of natural products is evolving fast. Accordingly, regulatory measures with potentially detrimental effects on treatment options highly relevant in phytotherapy might need to be reconsidered. Acknowledgements: Many thanks for fruitful discussions to D. Schrenk and J. Fahrner, RPTU Kaiserslautern-Landau, Germany.

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P521 Comorbidities and comedication in the treatment of functional gastrointestinal disorders – pharmacoepidemiological data on a herbal medicinal product

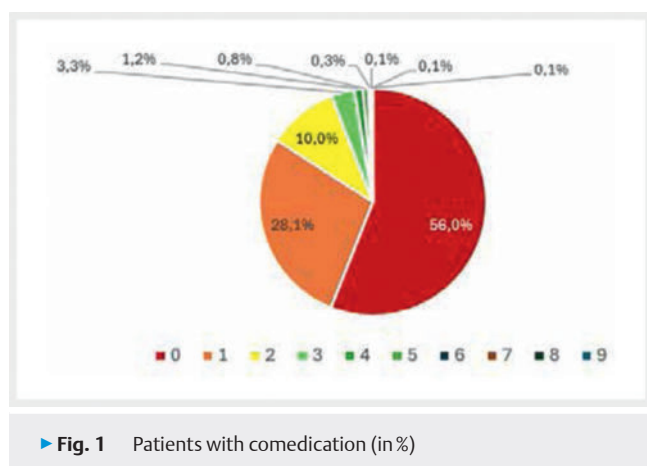
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Herbal Medicinal products (HMPs) are key in the treatment of Disorders of the Gut Brain Interaction (DGBI), which are often accompanied by comorbidities and also respective comedication. To assess these, real world data (RWD) for a

leading medicinal product in the field, STW 5 [2, 3] were captured from the PhytoVIS study [1], where RWD on the usage of HMPs from more than 20,000 patients were collected. The respective data sets were evaluated based on the ATC code. From the 1515 patients identified, 32.8% reported one or more comorbidities, most frequently hypertension (11.9%), metabolic disorders (9.0%), and back pain (3.6%). 56% reported no comedication, 28.1% taking one and 15.9% taking up to nine additional medicines (► Fig. 1). 45.6% of female and 39.9% of male patients, resp. 76.3% of those over 65 years of age reported co-medication. The ATC code C (cardiovascular system) accounted for 26.3%, A (alimentary system and metabolism) for 20.0%, H (systemic hormone preparations excluding sex hormones and insulin) for 12.8%, and N (nervous system) for 8.4% of the co-medication, to name the four most common ATC codes. Thyroid hormones (17.9%) and low-dose ASS (4.0%) were among the medicines most frequently mentioned. There was no evidence of interactions between STW 5 and the co-medication. Approximately onethird of the patients had a comorbidity, and almost half were receiving co-medication in addition to treatment with STW 5. The data thus provide a unique pharmacoepidemiological overview for a large cohort of patients with functional gastrointestinal diseases [4].



► Fig. 1 Patients with comedication (in %)

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P522 Genotoxicity studies of milk-thistle fruit (*Silybum marianum* fructus) dry extracts: no hints of mutagenicity

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Herbal medicinal products (HMPs) prepared from *Silybum marianum* are traditionally used mainly in the treatment of liver diseases and dyspeptic symptoms [1, 2]. The safety assessment of such HMPs with long standing traditional use require special attention to genotoxic effects [3]. A systematic “bracketing and matrixing” approach in line with the guidelines of the Herbal Medicinal Product Committee (HMPC) was chosen to provide a more comprehensive dataset covering extracts with different polarities of the extraction solvents [4, 5]. Previous genotoxicity studies of *S. marianum* extracts gave inconsistent results [1, 2]. Thus, additional data from Ames tests of extracts covering the full polarity range of extraction solvents may help to assess the safety of HMPs containing *S. marianum* extracts [4, 5]. Five dry extracts of *S. marianum* and a Silymarin concentrate, representing the whole spectrum of polarities of the extraction solvents (water – 60% ethanol (v/v) – 96% ethanol (v/v) – acetone – *n*-heptane) and the active ingredient Silymarin, were tested in the Ames test according to the OECD and HMPC guidances [4, 5]. When tested up to 5 mg per plate or up to the solubility or cytotoxicity limits, none of the extracts resulted in a significantly increased number of revertants. The results for the five extracts tested and the active ingredient Silymarin show no signs of mutagenicity, suggesting that extracts of *S. marianum* fructus have no genotoxic effects [5]. Furthermore, the data may help to resolve the inconsistency of the results to date [1].

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P523 The new ICH E11A guideline – an advantage for the development of herbal medicinal products for children?

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There is still a shortage of medicines for children, even though the present European regulation [1, 2] was supposed to facilitate their development. However, the approval of herbal medicinal products (HMPs) for children has become more tempting. Today, it has become clear that the aim must be to avoid large, placebo-controlled clinical trials in children wherever possible. With the guideline for pediatric extrapolation (E11A) [3, 4] published on August 27, 2024, there is now a binding guideline for the extrapolation from adult to pediatric patients. The new guideline was systematically analyzed with regard to the extent to which it can facilitate the approval of HMPs for children. Three questions are addressed in the guideline: How similar are (1) the disease, (2) the pharmacology of the drug, and (3) the response to therapy in adults and pediatric patients? The pediatric extrapolation is considered as a continuum: Accordingly, if there are major differences between adults and children, more data are required. A pediatric extrapolation plan is then derived, which lists any necessary studies. Here particular emphasis lays on the use of pharmacokinetic data, but also real world data (RWD) are in scope. While for most HMPs, but also for locally acting preparations and vaccines, no pharmacokinetic data can be collected, referring to the similarities between adults and children can be a way forward [4]. For this, also RWD can be a support. This underlines the importance of GA's initiatives to give more weight to RWD in pediatric extrapolation [2, 5]. Overall, the new guideline may give cause for optimism.

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P524 Marshmallow cough syrup – physical effects

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The use of the aqueous extract of marshmallow root for respiratory symptoms has been described for the first time by Pliny the Elder [1], prominent victim of the outbreak of Mt. Vesuvius in 79 AD. STW 42 (Phytohustil®), a cough syrup based on this extract, has characterized analytically [2, 3], as well as their anti-inflammatory effects [4–6], but still, their physical effects [7] are key for their fast and strong action in irritative cough [8]. To characterize these, physical parameters were studied in comparison to 5 other liquid cough preparations available in the German market. STW 42 and the other liquid cough preparations (coded 1–5) were studied by rotational viscosimetry and regarding their retention on a mucosal surface (EpiOral resp. ex vivo) after labelling with [¹⁴C]-galactose and repeated rinsing, mimicking the effect of swallowing saliva on the pharyngeal mucosa. The viscosity of the preparations tested was in a range of 482–26 mPas, ranking in order of decreasing viscosity STW 42 > 2 > 1 > 3 > 4. The order of retention was (in decreasing order) STW 42 > 4 > 1 > 5 > 2 > 3. Comparison of the viscosity values with epithelial retention data indicated that viscosity alone did not account for the retention on epithelial tissues. The results confirm the unique physical properties of marshmallow cough syrup and so underline its usefulness in irritative cough already described by Pliny the Elder and confirmed since then by a huge body of evidence.

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P525 3D Bioprinted hydrogels with alkannins/shikonins for wound healing

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Skin is the body's largest organ and serves as a protective barrier against the external environment. While minor wounds can heal naturally, deeper or chronic wounds often require medical treatment to prevent infection and long-term complications. Traditional methods like skin grafting have several limitations, including limited donor site availability and the risk of complications such as infection, inflammation, or donor site damage. Tissue engineering offers a promising alternative by developing scaffolds that support tissue repair, while avoiding common problems associated with conventional treatments [1, 2]. In this study, we explored the incorporation of plant-derived natural compounds into 3D-printed hydrogel scaffolds designed for wound healing applications. The composite scaffolds were fabricated using Gelatin Methacrylate (GelMA) and alginate, two soft materials recognized for their biodegradability and biocompatibility [3]. To enhance the scaffolds' therapeutic potential, we impregnated them with a mixture of alkannins/shikonins (A/S), bioactive molecules isolated from the roots of *Alkanna tinctoria*, which comprise the active pharmaceutical ingredients of the strong wound healing ointments HELIX-DERM® and Epouloderm®. A/S possess well established antimicrobial, anti-inflammatory, and wound-healing properties [4].

We compared two methods for incorporating A/S into the scaffold: embedding A/S during hydrogel preparation before 3D printing and immersion after printing. The scaffolds were tested *in vitro* for their cytocompatibility with fibroblasts, antibacterial activity, and drug release.

Preliminary results indicated that the drug loading method influenced the scaffold's overall performance and bioactivity. While further research is needed, this work highlights how natural compounds can be exploited in new wound healing materials and scaffolds.

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P526 *In vitro* approach to evaluate the potential of botanicals to cause hepatotoxicity and drug interactions: Milk thistle, yohimbe, and kava as case studies

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Herbal medicines and dietary supplements are frequently implicated in liver injury and botanical-drug interactions (BDIs), yet mechanistic understanding is limited. The Botanical Safety Consortium (BSC), a public-private partnership led by FDA, NIEHS, and HESI, is advancing safety assessment strategies for botanicals used in vitro and in silico tools.

This study aims to present a case study-based approach to assess hepatotoxicity and BDI potential of three botanicals, Milk thistle (*Silybum marianum*), Yohimbe (*Pausinystalia johimbe*), and Kava (*Piper methysticum*), using an integrated battery of in vitro assays.

The selected botanicals were evaluated for cytotoxicity, CYP3A4 inhibition/induction, metabolite formation, and transcriptomics in 2D and 3D human hepatocyte models. Kava exhibited strong CYP3A4 induction and formed reactive metabolites in metabolic activation assays, consistent with its suspected hepatotoxic risk. Yohimbe induced CYP3A4 mRNA without metabolite formation, marking the first report of its potential for BDI. Milk thistle, often considered hepatoprotective, showed glutathione conjugate formation from taxifolin even without metabolic activation—suggesting a redox-based mechanism possibly underlying its Nrf2-mediated antioxidant response. 3D models confirmed sub-cytotoxic CYP3A4 inhibition by Yohimbe and Kava and highlighted differential cytotoxicity across endpoints (LDH leakage, albumin production).

In conclusion, this study demonstrates the utility of integrated in vitro methods for botanical safety profiling and highlights the unique mechanistic fingerprints of each extract. Future efforts will focus on physiologically based pharmacokinetic (PBPK) modeling and clinical translation of these findings, especially for Yohimbe and Kava.

P527 Zebrafish-based phenotypic screening of herbal medicine to identify candidates for muscular dystrophy treatment

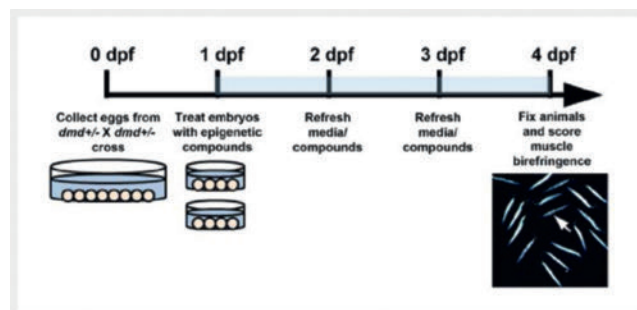
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Duchenne muscular dystrophy (DMD), caused by mutation in the dystrophin gene, leads to progressive muscle degeneration. In zebrafish, dystrophin is initially localized at myofiber ends near the myotendinous junction and later redistributed to non-junctional regions. Dystrophin-deficient zebrafish larvae exhibit necrotic muscle fibers, mononuclear infiltration, inflammation-associated fibrosis, and variability in muscle fiber size, closely mirroring the human DMD phenotype [1]. To identify potential therapeutic candidates, we performed a phenotype-based screening of 60 herbal medicines using the DMD zebrafish larvae. Zebrafish larvae (1 day post-fertilization, 1 dpf, N = 60 per group) were treated with herbal extracts at concentration of 40 µg/mL for 3 days by adding the extract directly to the culture plates. Muscle birefringence was analyzed by placing anesthetized embryos on a glass polarizing filter and

covering the embryos with second polarizing filter [2, 3] (► Fig. 1). One herbal formula (KMSO) and three individual herbal extracts (DR, CO, and PC) exhibited a trend toward ameliorating muscle damage, suggesting potential therapeutic relevance. These findings indicate that the identified herbal candidates may hold promise as therapeutic potential for muscular dystrophy. However, further validation in mammalian models is required to establish their efficacy and safety prior to clinical application.



► Fig. 1 Schematic diagram of the experiment

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P528 Labelling of food supplements targeting sleep disorders: current practices and challenges in the EU

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Quality sleep is a fundamental human need, crucial for overall health, and can be influenced by a wide range of factors. However, many individuals suffering from sleep disorders, particularly those with milder issues related to sleep quality, do not seek pharmacological treatment. Instead, they often self-manage their symptoms through dietary modifications, including the use of food supplements (FS). In the European Union (EU), the regulation of FS and Health Claims (HC) has been harmonized with the primary aim of supporting the functioning of the internal EU market and protecting consumers from misleading practices and potential health risks. This study aims to investigate the composition and labelling of FS targeting sleep disorders. The Slovenian branded foods database includes data on the composition and labelling of branded foods, including FS (N = 1,912; data collected in 2023). Data on ingredients were used to identify products targeting sleep disorders. Labelling practices involving HC were explored within the context of the current legislative framework. One of the most commonly used botanicals in these products is valerian (*Valeriana officinalis*). Although HC for valerian have not yet undergone scientific evaluation by the EFSA, its traditional and well-established use in treating sleep disorders is supported by herbal monographs (EMA, ESCOP) and 'on hold list' enables their use. The study results provide valuable insights to producers for responsible marketing practices and offer policymakers important information on the current market situation.

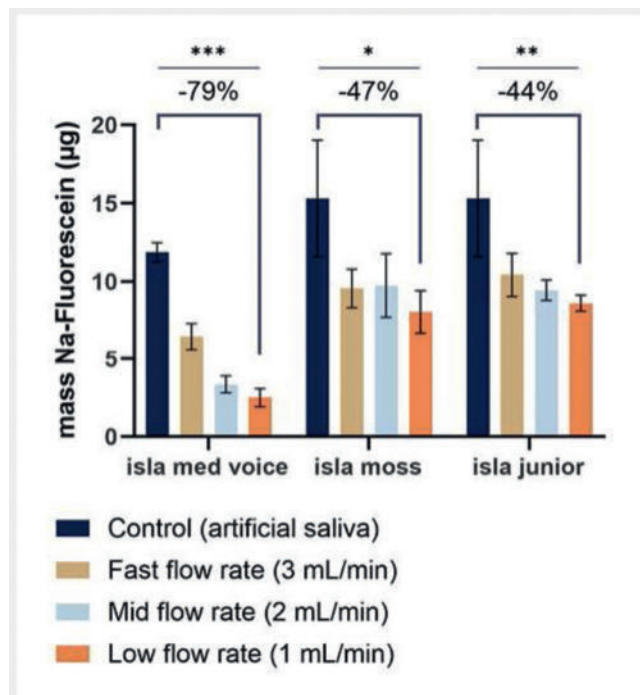
P529 Investigating the formation of a physical barrier by icelandic moss lozenges in vitro

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The common cold, predominantly caused by viral infections, is a widespread illness, for which preventative treatment options remain scarce. This study investigates the potential of Icelandic moss containing lozenges Isla moss[®], Isla junior[®], and Isla med voice[®] to act as a physical barrier against viral penetration. Different *in vitro* models were applied investigating lozenges dissolved in artificial saliva. A Franz diffusion cell was used investigating diffusion of sodium fluorescein (Na-fluorescein) and rhinovirus RV-14 through a layer of respective lozenge solutions [1]. Further, two plaque assay protocols in HeLa cells inoculating RV-14 were used evaluating preventive and containing properties of Isla med voice[®]. Comparing diffusion of Na-fluorescein after 60 minutes, the highest concentration (observed at lowest flow rate) of Isla moss[®] decreased diffusion by -47%, Isla junior[®] by -44%, and Isla med voice[®] by -79% compared to artificial saliva (► Fig. 1). Furthermore, dissolved Isla med voice[®] decreased RV-14 diffusion dose-dependently up to 99%. Additionally, applying an Isla med voice[®] solution before RV-14 inoculation on HeLa cells resulted in reduced plaque formation indicating formation of a preventive physical barrier. Lastly, treatment of HeLa cells with an Isla med voice[®] solution after RV-14 inoculation resulted in reduced plaques compared to the negative control suggesting a virus containing effect and less viral spreading. These findings demonstrate that Icelandic moss-based lozenges form a substantial physical barrier impairing diffusion of Na-fluorescein and rhinovirus RV-14. Further, Isla med voice[®] with its hydrogel complex revealed preventive and containing effects against rhinovirus RV-14 due to its physical properties.



► Fig. 1 Diffusion of sodium fluorescein through a cellulose mixed ester membrane covered with dissolved lozenge solutions in a Franz cell diffusion model after 60 min. Different lozenges were dissolved in artificial saliva depending on the sucking time until complete dissolution tmed voice=8.6 min, tmoss=6.4 min, tjunior

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P530 SAIRA – Explore Sustainable indoor Aeroponic cultivation and development of an Innovative anti-inflammatory plant extract of *Rhodiola rosea* – an endangered Alpine plant

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Rhodiola rosea L. (Crassulaceae), a perennial herb native to high-altitude alpine regions, is renowned for its adaptogenic and anti-inflammatory properties [1, 2]. Inflammation is involved in the development of neurodegenerative disorders, obesity and diabetes, which become, beside climate change and increasing world population, the major challenges for human beings of the 21st Century having significant effects on social and health systems. However, wild populations of *Rhodiola* are under increasing pressure due to overharvesting and habitat loss, leading to its classification as an endangered species [3]. SAIRA addresses the urgent need for sustainable *R. rosea* cultivation by investigating the potential of indoor aeroponics. We hypothesize that controlled-environment aeroponics can provide a viable alternative to traditional cultivation methods [4], ensuring a consistent supply of high-quality plant material while minimizing environmental impact by

- (1) optimizing aeroponic growth conditions for *Rhodiola* to maximize biomass production and bioactive compound accumulation.
- (2) developing and characterizing innovative green extraction methods for isolating key anti-inflammatory compounds.
- (3) evaluate the anti-inflammatory activity of the innovative *Rhodiola* extract using *in vitro* assays, focusing on the modulation of relevant inflammatory pathways.

The successful implementation of this project will contribute to the conservation of *Rhodiola* by reducing pressure on wild populations, provide a reliable source of raw material for the nutraceutical and might also phytopharmaceutical industries, and advance our understanding of the plant's nutritional and pharmaceutical potential. Furthermore, this approach offers a sustainable and scalable solution to produce valuable nutritional and medicinal plants, addressing both conservation concerns and the growing demand for natural health products.

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P531 eCommerce prospects on South African medicinal plants as ingredients in nutraceutical products

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Nutraceuticals are food-derived products that provide health benefits, including disease prevention and management [1]. The ingredients are derived from natural sources such as plants, fungi, algae, minerals, and animals [2]. In the last decade, medicinal plants have been used as ingredients in nutraceutical products due to their therapeutic benefits associated with their active phytochemicals [3]. In this review, the aim was to identify nutraceutical products sold on eCommerce platforms that include South African medicinal plants among their ingredients. The review also summarizes the phytochemical, nutritional, and bioactive properties of the medicinal plants identified in the nutraceutical products. An online search was performed across major South African eCommerce platforms to record available nutraceutical products, ingredient composition and their health benefits. The data related to the nutritional, phytochemical and clinical studies of the identified plant-based ingredients were extracted systematically from Google Scholar, PubMed and Scopus. Over 20 plant species were identified as ingredients in the nutraceutical products (► **Table 1**). The predominant products sold were dietary supplements, in form of capsules, powders and functional beverages such as teas and juices. Reported health benefits included stress and anxiety management, cold and flu relief, antidiabetic and anti-inflammatory effects. The review highlights the importance of phytochemical and nutritional profiling of South African medicinal plants and the need for bioavailability assessment of the active compounds as well as clinical studies on their safety to enhance their application in nutraceutical development. In addition, enforcing regulations on post-harvest handling, formulation, and dosage of plant-based nutraceuticals remains crucial to ensure high-quality products for consumers.

► **Table 1** Nutraceuticals from medicinal plant ingredients sold in eCommerce platforms

Health benefits	Plants species	Nutraceutical classification
Stress management and anxiety	Sceletium tortuosum	Capsule
Stress management and anxiety	Withania somnifera	Capsule
Stress management and anxiety	Aspalathus linearis	Capsule
Stress management and anxiety	Oenothera biennis	Capsule
Stress management and anxiety	Valeriana officinalis	Capsule
Appetite suppressant	Hoodia gordonii	Herbal tea
Colds and flu	Pelargonium sidoides	Herbal drink
Anti-inflammatory	Annona muricata	Herbal drink

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P532 Effect of a *Serenoa repens* extract on human primary hair dermal papilla cells isolated from male and female donors

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Human Hair Dermal Papilla Cells (HHDP) are strongly implicated in hair growth cycle. The aim of this study was to test a *Serenoa Repens* Extract (SRE; nominal value 90 % fatty acids; Euromed), on HHDP obtained from the scalp of 2 healthy donors, an adult man and a 66 years old woman. At different times following incubation with SRE, cells were tested using commercial assays.

On HHDP from male donor, cell viability (24–48 h of incubation) was strongly inhibited by high concentrations of SRE (100–1000 µg/mL). On the same cells, however, cell proliferation was significantly increased by SRE (incubation 72 h) at 10 and 20 µg/mL (+ 24 % and + 28 %, respectively), comparable to the effect produced by Epidermal Growth Factor (EGF) (+ 32 %) after 72 hours of incubation [1].

Using cells from female donor, SRE at 20 µg/mL (72 h incubation), slight increased cell viability, suggesting that this concentration promotes healthy conditions. SRE at 30–40 µg/mL had no effect on cell viability but a slight decrease was observed at 50 µg/mL.

On the same cells, proliferation was significantly increased by SRE (72 h incubation) at 20 µg/mL (+ 54 % compared with control cells). At 120 h however, we did not observe an increase of proliferation by SRE at any concentration with respect to controls.

We conclude that SRE (90 % fatty acids) has the potential to be a treatment of alopecia in both male and female patients. Further studies will be necessary to identify the mechanism of action on cell proliferation.

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P533 Improved sleep quality and daytime function through a multi-herbal nutritional supplement containing *Lactuca sativa* and *Melissa officinalis*

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Sleep disturbances are highly prevalent in modern societies and are often accompanied by impaired mood, cognitive dysfunction, and reduced quality of life [1, 2].

This exploratory, single-arm interventional study evaluated the effects of a novel nutritional supplement containing extracts of fresh *Lactuca sativa* L. herb,

Melissa officinalis L., L-Tryptophan, and Magnesium on sleep quality and daytime performance in 50 healthy adults with self-reported sleep disturbances. Over a 14-day period, subjects consumed one sachet nightly. Sleep quality (primary endpoint) and secondary outcomes related to mood, cognition, and physiology were assessed using validated questionnaires, diaries, wearables, and polysomnography (N = 10).

Sleep quality scores (SF-B/R) improved by 14 % overall ($p = 0.003$), with a greater benefit in highly anxious individuals (+ 37 %, $p < 0.001$). Sleep diaries revealed a 31 % reduction in awakenings and increased restedness (up to + 28 %, $p < 0.001$). PSG analysis indicated a 70 % increase in deep N4 sleep ($p = 0.042$) and a 26 % reduction in apnea-hypopnea index ($p = 0.11$). Exploratory assessments of wearable, sleep quantity, and salivary biomarkers yielded inconsistent results. Psychological tension (DASS-21) decreased up to 29 % and daytime mood improved (+ 23 %, $p = 0.014$). Cognitive performance increased by 13 % on executive function tasks ($p < 0.001$). The formulation was well tolerated with no serious adverse events.

These findings suggest that this supplement offers a promising, well-tolerated nutritional approach for improving sleep quality and daytime function, especially in individuals experiencing psychological strain.

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P534 Cognitive and emotional benefits of a herbal supplement with *Scutellaria baicalensis* and *Crataegus* extracts in stressed adults: a double-blind crossover trial

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Chronic stress impairs mood, sleep, and cognitive function through dysregulation of neurotransmitter systems such as GABA and dopamine [1, 2]. Plant-based supplements containing flavonoid- and polyphenol-rich extracts have shown potential in preclinical studies for restoring emotional balance and mental performance [3]. This randomised, double-blind, placebo-controlled, crossover study evaluated a chewable supplement containing *Scutellaria baicalensis* root (400 mg), *Crataegus laevigata* (Poir) D.C. (Rosaceae) fruit (40 mg), Magnesium, and Chromium in 35 healthy adults reporting elevated stress ($PSS \geq 13$).

Participants received both supplement and placebo for 15 days. Compared to placebo, the supplement significantly reduced trait anxiety (STAI trait, $p = 0.007$), total mood disturbance (POMS, $p = 0.032$), and anger-hostility ($p = 0.019$). A higher proportion achieved $\geq 30\%$ gains in WHOQOL social satisfaction scores ($p = 0.042$). Sleep duration increased after 15 days ($p = 0.037$), though subjective sleep quality effects were mixed. Under acute stress, serial 3's subtraction errors decreased post-dose ($p = 0.015$) and serial 7's accuracy improved ($p = 0.046$). Cognitive effects were less pronounced in non-stress conditions. These findings suggest that this multi-herbal supplement improves emotional wellbeing and cognitive resilience during stress. The observed effects are consistent with the proposed mechanisms of baicalin, wogonin, and chlorogenic acid [3–5].

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P535 Proapoptotic and antiproliferative effects of medicinal plant extracts in 2D and 3D cultures of breast, gastric, and colorectal cancer cells

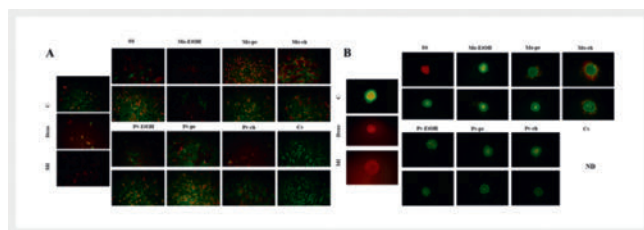
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Cancer remains the second leading cause of death worldwide, accounting for nearly 10 million deaths annually. This critical global burden highlights the urgency for new therapeutic strategies, among which phytotherapy stands out as a promising source of bioactive antitumor compounds [1, 2]. In this study, the antitumor potential of *Sapindus saponaria*, *Plukenetia volubilis*, *Moringa oleifera*, and *Caesalpinia spinosa* extracts was evaluated using 2D and 3D cultures of MCF7 (breast), AGS (stomach), and SW48 (colon) tumor cell lines. The extracts' effects on cell proliferation, apoptosis, mitochondrial membrane potential ($\Delta\Psi_m$), and cell cycle progression were assessed.

In 2D cultures, all extracts reduced cell proliferation, with *C. spinosa* (CS) and *S. saponaria* (SS) producing the most pronounced effects. CS extract notably increased Sub G1 cell population, indicating extensive DNA damage and apoptosis. In 3D cultures that simulate the *in vivo* tumor environment, SS maintained sustained activity, inducing early apoptosis and significant $\Delta\Psi_m$ disruption in AGS and SW48 spheroids (► Fig. 1).



► **Fig. 1** Effect of plant extract in viability/mortality of 2D (A) and 3D (B) MCF7 culture cells. SS: *Sapindus saponaria* saponins; Mo-EtOH: *Moringa oleifera* ethanol extract; Mo-PE: *M. oleifera* petroleum ether extract; Mo-CH: *M. oleifera* cycle hexane extract; Pv-EtOH: *Plukenetia volubilis* ethanol extract; Pv-PE: *P. volubilis* petroleum ether extract; Pv-CH: *P. volubilis* cycle hexane extract; CS: *Caesalpinia spinosa*; Doxo: doxorubicin; Mf: miltefosine. C-: untreated cells. ND: No data (complete destruction of spheroids). The upper panel in each Figure corresponds to a 200 µg/mL concentration, while the lower panel corresponds to a 100 µg/mL concentration.

$\Delta\Psi_m$ analysis confirmed that SS and CS primarily induced apoptosis through early mitochondrial membrane disruption, with low necrosis levels—suggesting a controlled, non-inflammatory mechanism of cell death. Cell cycle analysis revealed arrest and Sub G1 accumulation, confirming interference with cell division. Additionally, *P. volubilis* and *M. oleifera* exhibited selective cytotoxicity in colon cancer cells, indicating potential for colorectal cancer therapy.

These findings support the antitumor efficacy of plant-derived extracts via proapoptotic pathways and advocate for their further investigation as potential standalone or complementary agents in cancer treatment.

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P536 Investigation on the metabolome and potential application in cosmetics of different native apple varieties from Molise region (Italy)

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Apples (*Malus domestica* (Suckow) Borkh.) are among the most widely consumed fruits worldwide belonging to Rosaceae family and are a rich source of phytochemicals that may contribute to their health benefits [1]. The metabolome of many plants, such as apples from Molise region of Italy, is still unexplored. The main aim of this project is to characterize the metabolic profile of different native apple varieties from Molise (Fig. 1) using LC-MS techniques and to develop a cosmetic product derived from the selected varieties. LC-MS analysis revealed numerous polyphenols, primarily flavonoids, including catechin, procyanidin B2, cyanidin 3-O-glucoside, quercetin glycosides, and phenolic acids like chlorogenic acid. Qualitative analysis was performed manually and using online databases. Principal Component Analysis (PCA) of LC-MS data showed the separation of four distinct clusters and suggested metabolites that contribute to this separation. Metabolite abundances were then estimated by comparing their intensities across whole fruits and their tissues (pulp, peel, and seeds) separated during sample preparation. Since natural products may find use as nutraceuticals and cosmeceuticals thanks to their bioactivities [2], a green extraction process will be developed to formulate a cosmetic product based on the identified compounds from Molise apples. Bioactivity assays will be performed to assess the anti-inflammatory, antioxidant, and anti-aging properties of the extracts. This work, applied also to the waste of the selected fruits, will represent a potential valorization of these Molise products. LC-MS metabolomics showed the presence of bioactive polyphenols and the separation of the selected apple varieties through PCA, supporting further cosmetic exploration.

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P537 Approach to ensure quality of pharmaceutical care for cognitive impairment – integrative utilization of *Ginkgo biloba* L. leaf extract

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The prevalence of cognitive impairment like dementia is rising, making prevention and intervention increasingly important. According to the EMA/HMPC, refined and quantified *Ginkgo biloba* L. leaf extract is proven effective and safe in treatment [1]. To ensure the quality of pharmaceutical care in everyday practice, real-world data (RWD) are necessary to evaluate utilization, effectiveness and tolerability. Therefore, we analyzed patient-reported outcomes (PROs) from 191 patients with cognitive impairment regarding the utilization of *Ginkgo biloba* L. leaf extract (GBE), taken from the pharmaco-epidemiological database PhytoVIS.

Our results indicate that patients used GBE to treat chronic (41 %) or acute (20 %) symptoms, but also for prevention (39 %). Patients took GBE daily (>97 %) and for longer periods (>30 days: 86 %). Older patients used GBE significantly longer compared to younger patients ($p=0.002$, $r=0.228$). Daily doses were predominantly 120 mg (37 %) and 240 mg (35 %) – interestingly, men chose significantly higher doses than women ($p=0.021$, $r=0.168$).

The vast majority (>90 %) perceived a therapeutic benefit. Longer use of GBE (>30 days) was significantly associated with higher perceived effectiveness compared to shorter use ($p=0.005$, $r=0.207$). Although men took higher doses, they did not experience greater benefits. Self-reported tolerability was high, with 89.0 % not reporting adverse drug events (AEs).

Our findings suggest a favorable benefit-risk ratio of GBE for treatment and prevention of cognitive impairment, especially for long-term use. Regarding the quality of pharmaceutical care, it must be clarified how certain factors such as dosage, duration or frequency of application influence effectiveness and tolerability in the real world.

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P538 Clinical practice and evidence needs of clinical pharmacists regarding herb-drug interactions in China: a semi-structured in-depth interview study

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Research focusing on healthcare professionals' perspectives and practices on herb-drug interaction (HDI) in culturally distinct regions like China remains critically needed. [1] Despite this need, human-based HDI research continues to be severely limited. Our preliminary survey revealed that 37.5 % of clinicians

(the highest proportion) consult pharmacists when encountering non-clinical HDI evidence, underscoring pharmacists' central role in this domain. This study therefore examines Chinese clinical pharmacists' practical experiences with HDIs and their evidence requirements, while proposing strategies to enhance future research. We conducted semi-structured in-depth interviews with twelve Chinese clinical pharmacists, selected through purposive sampling until reaching data saturation. The interview guide was developed following comprehensive literature review, expert consultation, and preliminary interviews. Using NVivo 12, we performed thematic analysis of interview transcripts, identifying five core themes: (1) professional roles in HDI management, (2) knowledge acquisition methods, (3) approaches to addressing potential HDIs, (4) clinical implementation challenges, and (5) critical evidence gaps. Analysis revealed that pharmacists mainly depend on drug labels, clinical guidelines, and pharmacopeias, occasionally supplemented by limited preclinical (animal/cellular) or clinical studies. Participants consistently expressed strong demand for: (a) higher-quality clinical evidence, (b) mechanistically informative research with direct clinical applications, and (c) evidence-based frameworks to guide decision-making. Particularly urgent needs include developing systems to: (i) evaluate preclinical evidence quality, (ii) synthesize clinically useful data, and (iii) bridge the gap between basic research and clinical practice for improved HDI management in integrated medicine settings. The authors declared no potential conflict of interest.

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P539 Antibacterial activity and polyphenol content in italian propolis from polish Lozengers supplements

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Propolis extract has antibacterial, anti-inflammatory and antioxidant properties, which are primarily attributed to the presence of polyphenols, within its composition. However, its composition differs depending on the geographical origin of the raw material. This is a significant factor for patients considering the use of dietary supplements, given the persistent rise in interest in natural treatment methods. The quality of the raw material used to produce the Polish dietary supplement is not subject to the same level of control as medicinal products [1]. A series of analytical procedures were conducted on both a dry propolis extract and a dietary supplement used on the Polish market, encompassing microbiological analysis (reference strain *Streptococcus salivarius* (ATTC 13419), gas chromatography composition analysis (HS-SPME-GC/MS method), total polyphenol content analysis (Folin-Ciocalteu method), thermogravimetric analysis (TGA) and surface analysis by scanning electron microscopy (SEM/EDS). No growth inhibition zones were observed for the reference strain in the selected sample containing propolis. The morphology of the dry extract was found to be heterogeneous, and the arsenic content was higher than that declared by the manufacturer. The total polyphenol content of the studied propolis was lower than the mean total polyphenol content of European propolis as reported in the extant scientific literature [2]. It has been suggested that the propolis extract, which has been comprehensively documented, may be susceptible to degradation [3]. The most probable reason for this degradation is the inappropriate storage of the propolis extract, both during transport and during the technological production process, which requires further investigation.

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P540 Phytochemical composition and *in vitro* hepatoprotective potential of a detoxifying herbal formula used in highland communities

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Traditional herbal detoxifying remedies are widely used in highland communities to mitigate the adverse effects of pesticide exposure. This study aimed to investigate the phytochemical composition and *in vitro* antioxidant and hepatoprotective potential of a detoxifying herbal formula (Formula 04), comprising *Thunbergia laurifolia* Lindl and *Embelia sessiliflora* (Vahl) Blume. The aqueous extract was prepared by decoction and analyzed using thin-layer chromatography (TLC), which revealed the presence of phenolic and flavonoid compounds, including caffeic acid as a major constituent. Antioxidant activity was assessed using DPPH and superoxide radical scavenging assays. The extract's effects on hepatic stellate cells (LX-2) were evaluated using sulforhodamine B (SRB) cell viability and apoptosis assays. Formula 04 demonstrated significant antioxidant activity, with an IC₅₀ of 76.0 ± 4.9 µg/mL in the DPPH assay. *In vitro* testing showed that the extract induced early apoptosis in LX-2 cells in a dose-dependent manner, without causing substantial late-stage cytotoxicity, suggesting a potential antifibrotic effect. These findings support the traditional use of this herbal formula for detoxification and highlight its potential as a safe, plant-based intervention with antioxidant and hepatoprotective properties. Further studies are warranted to confirm its mechanism of action and potential for development as a standardized product [1].

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P541 Evaluation of anti-inflammatory activity of root extracts from different *Arctium* species in skin cell models

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Burdock root (*Arctii radix*) is a medicinal plant traditionally used to treat seboreic and inflammatory skin conditions. The plant substance can be sourced from three species of *Arctium*: *A. lappa*, *A. tomentosum*, and *A. minus*. Nevertheless, their biological activity has not yet been compared [1]. The present study aimed to compare the chemical composition of 70 % ethanolic extracts prepared from the roots of *A. lappa*, *A. tomentosum*, and *A. minus*, collected in the Podkarpacie region of Poland. Subsequently, their impact on the inflammatory response of skin cells, fibroblasts (NHDF) and keratinocytes (HaCaT), was evaluated. The effect on cell viability was examined using the MTT assay. The acute and chronic inflammatory response was induced by stimulating fibroblasts with lipoteichoic acid from the cell wall of *Staphylococcus aureus* and keratinocytes with a mixture of TNF- α and IFN- γ , respectively.

The principal chemical compounds identified by UHPLC-DAD-MSn analysis were phenolic acids, primarily dicaffeoylquinic acids. The extracts at concentrations below 200 μ g/mL did not exert a detrimental effect on skin cell viability. Furthermore, all extracts exhibited a strong and concentration-dependent reduction in chemokine 8 secretion by both fibroblasts and keratinocytes. The extracts also reduced interleukin 6 release by fibroblasts, while its secretion by keratinocytes was increased by *A. lappa* and *A. tomentosum* root extracts.

Despite chemical differences, the extracts exhibited comparable biological activity. Further research and standardization are needed to confirm their equivalence as sources of *Arctii radix*. Their anti-inflammatory properties suggest potential use in treating inflammatory skin conditions like atopic dermatitis.

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P542 Safety evaluation of silver fir (*Abies alba*) branch wood extract (Belinal®) in healthy human subjects

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Silver fir (*Abies alba*) branch wood extract (Belinal®) is rich in bioactive polyphenols, particularly lignans, and has demonstrated various pharmacological effects, including cardioprotective, antiatherosclerotic, antidiabetic and antioxidant activities. Although the extract has a history of traditional use, its toxicological safety in humans has not yet been rigorously evaluated [1, 2].

This study aimed to assess the potential hepatotoxic and nephrotoxic effects of Belinal® in healthy human subjects. Fifteen adult volunteers received 900 mg/day of the extract over a 14-day period. Liver and kidney function were monitored by measuring eight biochemical serum parameters (AST, ALT, gamma-GT, alkaline phosphatase, total bilirubin, urea, creatinine, uric acid) at three time points: baseline, pre-treatment, and post-treatment.

No statistically significant changes were observed in any of the parameters during the test period. All values remained within the reference range, and no adverse events were reported. Although minor fluctuations were noted during the comparative period, these were not treatment-related and likely reflect normal physiological variability.

The findings provide direct evidence that Belinal® does not appear to adversely affect hepatic or renal function under the tested conditions, supporting its good safety profile as a food supplement. These results complement previous safety and effectiveness data and contribute to the toxicological characterization of silver fir extracts for human use.

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P544 Comparative assessment of the antioxidant capacity of Domat olive leaf and Sari Yaprak olive leaf

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Oxidative stress can cause several degenerative conditions, including inflammation, cardiovascular diseases, and cancer. Organisms have inherent mechanisms to reduce oxidative stress, but it is not entirely effective. Thus, they can benefit from additional antioxidants. Many fruits, vegetables, and plant leaves have antioxidant properties. Olive leaves are particularly interesting because they have been used in traditional treatments throughout the Mediterranean [1], and their health benefits have been supported by scientific studies [2]. On the other hand, different olive leaves cultivars showed different antioxidant activity [3]. This presentation will cover the antioxidant capacity of two different *Olea europaea* Domat olives cultivars and Sari Yaprak olive leaves collected from Izmir, Turkey. Domat olive leaves showed higher antioxidant capacity when compared with 21 other olive cultivars [3]. The Sari Yaprak olive cultivar is exclusively grown in Turgutlu and Manisa, Turkey. In addition, how the antioxidant capacity of sari yaprak olive leaves was affected by geographical features was tested. Different cultivars of olive leaves were extracted with 80 % methanol. Their antioxidant capacities were compared with DPPH radical scavenging activity, CUPRAC (cupric reducing antioxidant capacity), and TOAC (total antioxidant capacity) assays. Their phytochemical properties were compared with total phenolic content and total flavonoid content. Sari Yaprak olive leaves showed higher total flavonoid content than Domat olive leaves, whilst Domat olive leaves had higher antioxidant activity on CUPRAC than Sari Yaprak olive leaves. Furthermore, environmental conditions such as altitude, climate, and light affected the total phytochemical assays and antioxidant activities of Sari Yaprak olive leaves.

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P545 An old system for new applications: perspectives of the yeast-3-hybrid screening for natural product research

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Identifying protein targets of small molecules—commonly referred to as ‘target fishing’—is challenging and typically requires intensive effort [1]. The process involves investigation of numerous possible interaction partners generated by high-throughput screening techniques, followed by validation of promising candidates. This demands a multidisciplinary approach and a high level of technical expertise. As an alternative to classical affinity-based proteomics, the yeast

three-hybrid (Y3H) system provides a valuable strategy for target deconvolution in living cells. First introduced by Licitra and Liu in 1996 [3], this method offers unique advantages, particularly for detecting interactions under physiological conditions. In our group, we have implemented an optimized version of the Y3H system and successfully used it to reveal a previously unidentified protein target of the widely prescribed contraceptive ethinylestradiol [4]. Natural products have drawn considerable scientific attention due to their biological properties. However, despite this interest, our understanding of their direct interactions with proteins remains limited. While small molecules can have significant biological effects, their precise protein targets and mechanisms of action often remain unidentified. Yet, this knowledge is essential for evaluating a compound's potential as a therapeutic agent [2]. Frequently, the key to explaining a compound's cellular impact lies in uncovering the molecular pathways it influences. We are currently employing an improved Y3H screening to explore protein targets of natural products, and are working to expand its capabilities to improve its adaptability and effectiveness in the context of natural product research.

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P546 Evaluation of a nanoformulation's chronic and pre-chronic toxicity based on natural extracts for potential use in treating obesity-associated complications

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An oral nanoformulation (ONF) based on natural extracts (OBE100); ursolic acid, oleanolic acid, and ursolic acid lactone, obtained by hexane: methanol: water 4:3:1 (v/v) extraction from *Eucalyptus tereticornis* Sm. (Myrtaceae) leaves reduce metabolic alterations in a diet-induced obese mouse model [1]. The work aims to develop preclinical safety and biological efficacy tests for the ONF. The findings of acute oral toxicity, oral toxicokinetics, and repeated doses of oral toxicity for 28 days indicate that the compound ONF is safe based on clinical and paraclinical evaluation and histopathological analysis. To continue evaluating the in vivo safety profile of the ONF, we conducted sub-chronic toxicity studies for 90 days and a combined chronic toxicity/carcinogenicity study. These studies were carried out according to OECD guidelines [2]. In the sub-chronic toxicity study, the histopathological analysis of the stomach, liver, spleen, kidneys, small intestine, and testicles did not show any representative lesions between groups or associated with toxicity, providing strong evidence of the compound's safety. Paraclinical tests and clinical follow-up did not present any abnormalities, further reinforcing the safety of the compound. For the combined chronic toxicity/carcinogenicity study, the following organs were taken: adrenal glands, spleen, brain, cerebellum, heart, esophagus, stomach, salivary glands, liver, small intestine, colon, lymph node, eyeball, pancreas, skin, lung, kidneys, uterus, testicles, gallbladder, ovaries,

epididymis, seminal vesicle, prostate. A total of 2320 tissues are being processed. The results of the clinical evaluation, blood chemistry, and blood count did not show any toxicity associated with the treatments. No histopathological or neoplastic lesions have been observed.

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P547 *Arctium lappa* (burdock) watery extract multifaceted effect on skin health: A transcriptomic analysis

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Burdock (*Arctium lappa* L.) is traditionally recognized as a prebiotic [1] but its molecular effects on the skin remain poorly understood. This study aims to elucidate the molecular mechanisms by which a burdock's watery extract-rich in bioactive compounds [2], including inulin and antioxidants- influences human keratinocytes. We treated HaCat cells with the burdock extract (1 mg/mL) or a vehicle control for 4.5 hours, followed by RNA extraction, RNAseq and DESeq2 [3, 4] analyses. A functional enrichment analysis, utilizing Gene Ontology (GO) and KEGG, was performed on the differentially expressed genes (log₂FC > |1|). GO revealed significant biological process enrichment, particularly those related to ribosome biogenesis and protein translation, indicating a robust impact on the protein synthesis machinery of skin cells. Changes in mitochondrial ATP synthesis and aerobic electron transport suggest enhanced energy production. KEGG pathway analysis highlighted enrichments in key signalling pathways, implicating potential modulation of cell growth, differentiation, and stress responses. These modulations suggest possible benefits in wound healing and protection against environmental stressors. Furthermore, the enrichment of pathways related to immune processes, including complement and coagulation cascades, suggests that burdock extract may exert immunomodulatory effects. It also demonstrated modulatory effects on mineral absorption pathways and signalling pathways regulating stem cell pluripotency, suggesting potential for enhanced nutrient uptake and regenerative capacity. Overall, this study provides a comprehensive molecular basis for the diverse benefits of burdock extract, reinforcing its role as a promising agent for enhancing skin health and providing protective effects against environmental stressors.

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P548 Matrix and nanoformulation-dependent bioavailability of cannabinoids in the Caco-2 Model

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Phytocannabinoids are bioactive plant-derived compounds with promising therapeutic potential [1], but their oral bioavailability remains limited and variable [2]. Understanding their absorption mechanisms is essential for optimizing formulation strategies and predicting pharmacokinetic behavior.

The bioavailability of various cannabinoids (10) was assessed *in vitro* using the Caco-2 cellular model. Permeability coefficients (Papp) were determined for individual cannabinoids, both in pure form and with added cannabis-derived matrices. Neutral cannabinoids showed significantly increased permeability in the presence of terpenic matrix components, while cannabinoid acids did not exhibit matrix-induced permeability changes. Additional analyses assessed cannabinoid distribution across the apical medium, Caco-2 cells, and basolateral compartment. Neutral cannabinoids primarily accumulated in cells (~57%), whereas acids remained mostly in the apical medium with minimal cellular uptake (~7%). In a follow-up experiment focused on nanoformulation, only cannabidiol (CBD) and its acid (CBDA) were tested. Nanoformulated CBD showed markedly reduced accumulation in Caco-2 cells, reaching levels similar to CBDA, likely due to micelle formation. Interestingly, CBDA maintained better basolateral passage even in nanoform, suggesting different intracellular processing mechanisms.

These findings highlight the importance of chemical structure, formulation, and matrix effects in modulating the intestinal absorption of phytocannabinoids.

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P549 Semisolid formulations with therapeutic potential: development and stability study of cream and emulgel based on brazilian medicinal plants

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Medicinal plants are a relevant source of bioactive compounds with potential applications in drug development. Species such as *Pterodon pubescens* Benth and *Cordia verbenacea* DC, native to Brazil, have shown anti-inflammatory and antinociceptive activities [1, 2] and synergistic effects when associated in formulations [3]. Thus, the aim of this project is to develop semisolid formulations (cream and emulgel) containing ethanol extract of *P. pubescens* (EEPP) and essential oil of *C. verbenacea* (EOCV) determining preliminary stability. The cream was prepared using a modified nonionic formulation from the Brazilian Pharmacopoeia [4], while the emulgel followed a previous study of Takayama et al. (2022), also with modifications [5]. Both formulations were made by heating the oily and aqueous phases to 75 °C, then mixing them. Considering the emulgel, the gel phase containing Natrosol® was incorporated into the emulsion after this step. EOCV and the EEPP were incorporated into the bases after cooling. Stability was assessed under three conditions: stability chamber

(40 °C ± 2 °C, 70 % humidity), refrigerator (5 ± 2 °C), and room temperature. Samples were analyzed for macroscopic appearance, centrifugation test, and pH over 15 days. Results indicated that the cream remained stable, with no phase separation, color or odor changes, and a pH variation of less than 10 %, while in the emulgel formulation, phase separation was observed from the third day of analysis. Further studies are necessary to verify permeation and the *in vivo* efficacy, nevertheless, these findings provides a promising formulation for the treatment of inflammatory conditions.

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P550 Hot melt extrusion as a strategy to improve formononetin bioavailability and enable hydrogel formulation

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Formononetin, an isoflavonoid compound, exhibits agonistic activity towards estrogen receptors, thereby modulating nuclear signaling pathways involved in the production of structural proteins such as elastin and collagen [1]. However, its therapeutic application is limited by its low aqueous solubility, which significantly reduces its bioavailability. Improving the solubility of formononetin may enable the development of an effective hydrogel formulation with regenerative and moisturizing properties. The aim of this study was to obtain a system with improved solubility of formononetin by combining it with hydroxypropyl-β-cyclodextrin (HPβCD) and Kollidon® VA 64 using a hot melt extrusion (HME) process, and to evaluate its potential for hydrogel-based delivery. The extrudate was characterized using thermogravimetric analysis (TG), differential scanning calorimetry (DSC), and X-ray powder diffraction (XRPD), confirming the formation of an amorphous dispersion of formononetin. The obtained HME system was then incorporated into a hydrogel formulation composed of gelatine, carboxymethylcellulose (CMC), arabic gum, and glycerine. The final hydrogel was evaluated in terms of formononetin release kinetics (using Franz diffusion cells), mechanical strength, and swelling capacity. The formulation demonstrated suitable mechanical properties, improved release and solubility of formononetin, and favorable water absorption. The application of HME with HPβCD and Kollidon® VA 64 enabled a significant increase in formononetin solubility and the development of a stable hydrogel formulation with potential for skin therapy and regeneration.

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P551 Characterisation of the honokiol derivative LRK071 as a dual-specific RXR agonist and PDE4 inhibitor

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Retinoid X Receptor (RXR), a ligand-activated transcription factor, and Phosphodiesterase 4 (PDE4), an enzyme that degrades cyclic AMP (cAMP), regulate key physiological processes, including immune response, inflammation, and cell differentiation [1, 2]. Consequently, RXR agonists and PDE4 inhibitors have been explored for various therapeutic applications. Despite their distinct mechanisms, their converging effects suggest potential synergy, which could be leveraged for treatments such as promoting remyelination in neurodegenerative conditions [3, 4]. In this study, the honokiol derivative LRK071 was identified as an RXR agonist following a screening of 55 neolignans using nuclear receptor luciferase assays. LRK071 exhibited increased specificity for RXR over other nuclear receptors compared to its parent compound, honokiol. Its RXR agonism was further validated in THP1 macrophages by the upregulation of RXR homodimer and RXR:RXR heterodimer-driven target gene expression using RT-qPCR. In addition, LRK071 showed potential as a PDE4 inhibitor by increasing cAMP levels in a concentration-dependent manner in an EPAC-based FRET biosensor assay [5]. No such effect was seen for the marketed RXR agonist, Bexarotene, indicating the effect was RXR-independent. Further characterization using a CRE-Luciferase assay confirmed the ability of LRK071 to induce the anti-inflammatory cAMP-PKA-CREB pathway, comparable to known PDE4 inhibitors. In conclusion, our data suggest that LRK071 is a dual-specific RXR agonist and PDE4 inhibitor with potential for synergistic therapeutic applications.

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P552 NFDI4Chem: digital solutions for modern chemistry research

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NFDI4Chem is the chemistry consortium within the German National Research Data Infrastructure (NFDI) [1], an initiative to systematically manage and make research data FAIR—Findable, Accessible, Interoperable, and Reusable [2]. The consortium supports the digital transformation of chemistry worldwide by providing community-driven services, tools, and training for sustainable research data management (RDM).

At the core of NFDI4Chem's service portfolio are domain-specific solutions tailored to the needs of chemical researchers [3]. These include support for a smart lab environment, such as the opensource Electronic Lab Notebooks (ELN) Chemotion, chemistry-specific data repositories, e.g. nmrXiv [5], and a Terminology Service offering standardised ontologies to enhance data interoperability. The consortium also runs a Knowledge Base with best-practice guidance, a Search Service to aid in data discovery, and a Help Desk providing direct support to researchers. NFDI4Chem works closely with partners across Germany and engages actively with international initiatives to ensure compatibility with global standards [3]. This includes collaboration with IUPAC, the Research Data Alliance (RDA), and integration into European efforts such as the European Open Science Cloud (EOSC). By fostering international alignment, NFDI4Chem ensures that data produced within Germany's chemistry community can be shared and reused globally, facilitating cross-border scientific collaboration. With a strong focus on enabling open science practices, NFDI4Chem empowers researchers to manage and publish their data following the FAIR principles—enhancing reproducibility, accelerating discovery, and laying the foundation for a more connected and data-driven future in chemistry [4].

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P553 Aqueous natural extract from *Chlorella vulgaris*: a new functional micro delivery system for healthy applications

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Chlorella vulgaris, a microalga rich in bioactive compounds, has potential applications as natural functional ingredient in nutraceuticals, cosmetics and as a biostimulant in agriculture [1]. A high functionality aqueous extract from *Chlorella vulgaris* was produced, during the “MIGLIORARE” and Agritech National Research Center projects activities; however, issues like instability, handling challenges, and reduced water solubility, likely due to extraction procedures, limit its applications. To address these limitations, a spray drying process was used to convert the extract into a stable microparticulate powder. Inulin [2] and Sodium carboxymethylcellulose were used as a carrier and coating

hydrocolloid, respectively, to facilitate microparticle formation. The product was characterized for process yield, extract loading efficiency, morphology, dimensional properties, and water dispersion behavior to evaluate its potential in various applications. The spray-dried microparticles exhibited a high process yield of over 90 % and an extract loading efficiency close to 100 %. Morphological characterization of the solid state showed that the resulting microparticles were predominantly spherical with a micrometric size distribution. The microparticles, when dispersed in water at high concentrations, demonstrated the ability to form a colloidal system with particle sizes reduced to below 1 micron, transitioning from the micro to the nano scale (below 0.5 microns). This colloidal system significantly enhances the ingredient's functionality. The nanometric colloidal system can improve permeation, dissolution rates, and interaction with the environmental system thus enhancing efficacy. The transformation of the *Chlorella vulgaris* extract into spray-dried form presents multiple functional advantages. The technological approach and the development of this delivery system could significantly enhance the efficacy of *Chlorella vulgaris* extract in diverse fields, providing a versatile platform for future healthy applications.

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P554 Design of experiments to develop and optimise the nanocarriers: the case of vesicles loaded with cannabidiol and coated with chitosan

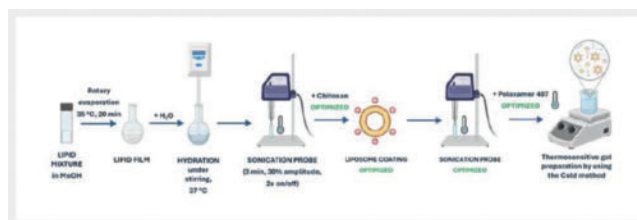
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The application of statistical analysis in the early stages of research allows quality to be built into the product from the outset, following a Quality by Design (QbD) approach. In formulation development, particularly for nanocarriers, the traditional “one factor at a time” method still dominates, whereas Design of Experiments (DoE) offers a more rational and efficient approach [1, 2]. In this study, chitosan-coated nanovesicles loaded with cannabidiol (CBD), a non-psychoactive phytocannabinoid with anti-inflammatory and neuroprotective properties, were embedded in a thermosensitive gel for topical ocular administration. A preliminary screening phase was conducted to evaluate the effect of three independent variables (% chitosan, coating time and % poloxamer 407) on five dependent variables: gelation time at 34 °C, vesicle size, polydispersity index (PDI), zeta potential and zeta potential change upon interaction with mucin. The aim was to identify the most influential factors for subsequent optimisation. Optimization was carried out using the Doehlert design, a versatile and efficient experimental design that allows three factors to be evaluated simultaneously at different levels, giving greater resolution to the most critical variables. At this stage, coating time was fixed, while sonication time was introduced as a key factor to reduce vesicle size and polydispersity. The final model was validated by performing four experiments. The optimised formulation showed a vesicle size of 181.6 ± 3.1 nm, a PDI of 0.160 ± 0.015 , a zeta potential of 12.31 ± 0.97 mV and a gelation time of 16 s. This approach allowed efficient

and precise development of the final formulation from nanovesicles to gel [3] (► Fig. 1).



► **Fig. 1** Batch production of CBD-loaded nanovesicles and thermosensitive gel formulation.

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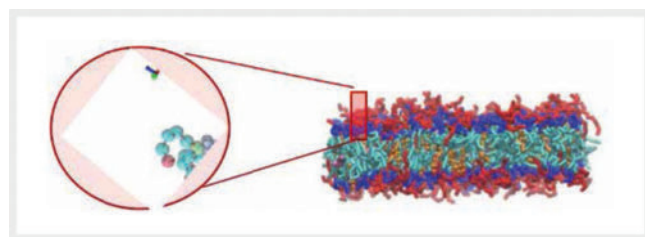
P555 Design and simulation of nanodrug delivery systems via Martini 3 Coarse-Grained molecular dynamics: the case of vesicles containing Tween20

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Vesicles are self-assembled nanocarriers composed of lecithins and cholesterol, capable of encapsulating hydrophilic, hydrophobic, and amphipathic drugs. They offer several advantages over conventional formulations, including improved therapeutic efficacy, enhanced drug stability, reduced toxicity, and full biodegradability, making them ideal for drug delivery. In recent years, vesicles have been explored as promising systems for delivering natural compounds [1, 2]. The inclusion of polysorbates (Tweens), such as polyethoxylated sorbitan esters, enhances vesicle properties by increasing drug loading, enabling ultra-deformability, and facilitating active brain targeting via receptor-mediated transcytosis [3, 4]. Computational tools are increasingly used in formulation design to provide mechanistic insights into excipient-drug interactions, allowing early identification of potential issues and improving the efficiency of the development process [5]. Despite this, computational approaches in nanoformulation are still emerging. In this study, membrane vesicles with and without Tween 20 (T20) were investigated using molecular dynamics simulations with a coarse-grained resolution based on the Martini 3 force field. A new Martini 3 model of T20 was developed and validated using structural parameters, such as end-to-end distances, radius of gyration, and micellar behavior. The validated model was then incorporated into a lipid bilayer composed of phosphatidylcholine and cholesterol to study its behavior within vesicle membranes. Molecular dynamics simulations were used to assess membrane properties, including mechanical characteristics, and to compute the free energy barriers associated with drug adsorption into the membrane. This work provides a found-

data for the rational design of deformable vesicles containing T20, supporting optimization of drug loading and release profiles (► Fig. 1).



► **Fig. 1** Vesicle membrane system containing Tween 20. The magnified area illustrates the coarse-grained Martini 3 representation of the surfactant within the lipid bilayer.

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P556 Pharmacokinetic investigation of 1,8-cineol after oral administration: correlation of blood and breath concentration to establish non-invasive kinetic quantification

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This biomedical study investigates the pharmacokinetics of 1,8-cineol from a dietary supplement after oral administration in adults. The aim is to establish a correlation between blood and breath concentrations in order to establish a non-invasive measurement method that makes it possible to quantify 1,8-cineol levels in the body without invasive blood sampling. The datasets established in this way will subsequently be used to develop and evaluate a physiologically-based pharmacokinetic (PBPK) model for 1,8-cineol (PK-Sim®, part of the OSP Suite). This model will enable the prediction of drug exposure in pediatrics based on age-related physiological differences, by extrapolating adult data. The aim is to determine appropriate dose recommendations for pediatric patients. The ICH guideline E11A, which deals with the extrapolation of data for dose determination for pediatric patients, serves as guidance [1]. The pre-study methodological validation for determining blood concentration by head-space GC-MS, as well as the sampling and measurement of breath samples, has been validated according to ICH guidelines [2]. Blood and breath samples are currently under analysis and final data will be presented soon.

The introduction of breath analysis as a non-invasive measurement method could significantly enhance patient comfort while providing new insights into the pharmacokinetics of 1,8-cineol. By developing a PBPK model, we aim to bridge knowledge gaps in pediatric dosing for herbal medicinal products (HMPs). This approach could contribute to a better understanding of pharmacokinetics in children, reduce the need for pediatric clinical trials, optimize drug development, and increase the availability of safe, evidence-based HMPs for pediatric use.

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P557 Target identification for natural products inhibiting oncogenic ERK/AKT signaling in malignant melanoma

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Malignant melanoma is the deadliest type of skin cancer driven by high mutation rates arising in both MAPK/ERK and PI3K/AKT signaling pathways. While targeted therapies show initial promise, drug resistance develops quickly, leaving patients with limited options. Novel treatments are therefore urgently needed.

As part of a broader drug discovery effort, we conducted two screening campaigns: one using our in-house library of 2,576 crude extracts, and another one leveraging large libraries of over 25,000 pure compounds from both natural and synthetic origin. Using a specifically developed high-content screening (HCS) assay that reports on downstream ERK and AKT activity, we identified 42 active compounds with IC₅₀ values below 20 µM.

The current challenge focuses on identifying the molecular targets of the most promising hits – an essential step toward understanding their mode of action and therapeutic potential. However, target deconvolution remains a significant challenge, particularly for phenotypic assays. To address this, we developed a complementary strategy to explore compound-target interactions that includes computational methods, thermostability-based binding assays and selective kinase activity assays. Key kinases of the ERK-AKT network were produced using different cloning techniques and heterologous expression systems for testing in these assays. Challenges encountered in target identification and first insights into potential targets will be presented. Ultimately, we aim to advance our newly discovered inhibitors into lead compounds for future drug development [1, 2].

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P558 Development and optimization of quercetin-loaded contact lenses for sustained anti-allergic drug delivery

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Quercetin is known for its potent anti-inflammatory and anti-allergic properties. [1] However, its clinical application in ophthalmology has been limited due to challenges in delivery and bioavailability. Thus, this study aimed to develop contact lenses containing naturally derived anti-allergic agents and to optimize condition for therapeutic application. Hydrogel (hilafilecon B), and silicone hydrogel (somofilcon A, delefilcon A) lenses were used as drug delivery platforms. Quercetin solution was prepared in phosphate-buffered saline (pH 7.4), and each lens was immersed in quercetin solution at different concentrations (50 µM, 100 µM, and 200 µM) for 0.5, 1, 2, 4, and 8 hours, followed by drying. Drug release from quercetin-loaded contact lenses was assessed at corneal temperature for a total of 120 hours. Quercetin loading amount and efficiency increased with immersion time regardless of lens material. At lower concentrations, hilafilecon B and delefilcon A showed higher loading efficiency compared to somofilcon A. At 200 µM with 8 hour-immersion, all materials exhibited loading efficiency above 70 %. At corneal temperature, hilafilecon B showed a higher cumulative release compared to delefilcon A. However, delefilcon A demonstrated superior release efficiency regardless of temperature. Both hilafilecon B and delefilcon A lenses exhibited cumulative quercetin release above 30 µM at corneal temperature. This study established optimal conditions for anti-allergic contact lenses with quercetin immersion in a 200 µM solution for 1 hour. The lenses demonstrated sustained drug release over 120 hours at corneal temperature without burst initial release, suggesting prolonged therapeutic potential. These findings highlight the quercetin's potential for further clinical evaluation and optimization of therapeutic applications [2].

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P559 Hazelnut skin extract in cosmetics: a multi-functional ingredient with antioxidant, preservative, and UV-boosting properties

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Agricultural by-products are rich in bioactive compounds and are increasingly explored as sustainable materials for cosmetic formulations [1]. Their potential use in cosmetics can turn these waste materials into valuable resources, meeting the growing demand for innovative ingredients from both the industry and consumers. Hazelnut (*Corylus avellana* L.) skin, a by-product of the roasting process, is rich in polyphenols, such as proanthocyanidins, with antioxidant and antimicrobial properties [2]. In this study, a chemically characterized hazelnut skin extract was incorporated into an oil-in-water (O/W) cosmetic emulsion to assess its stability and functional performance. The formulation was stored at 25 °C for six months and subjected to thermal shock cycles (4–50 °C every 24 h). Physicochemical parameters (pH, centrifugation, rheological behaviour, and zeta potential) were monitored. The antioxidant and preservative efficacy of the extract-loaded formulation was evaluated using the DPPH assay and a

Challenge test. Additionally, the extract ability to enhance UV protection was assessed by in vitro SPF determination (transmittance method). The extract-based emulsion demonstrated excellent physicochemical stability under stress conditions. The natural ingredient synergized with synthetic preservatives, enhancing antimicrobial efficacy, and slightly increased SPF, contributing to broad-spectrum photoprotection. These findings highlight the potential of hazelnut skin extract as a sustainable, multifunctional ingredient for skincare formulations, offering antioxidant protection, UV enhancement, and natural preservation.

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P560 COCONUT 2.0: The Collection of Open Natural Products Database

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Natural Products (NPs) are bioactive small molecules synthesized by living organisms, long recognized for their pharmaceutical and industrial potential. Despite numerous databases, a comprehensive online resource compiling digitally documented NP structures in one place was lacking. The COLleCtion of Open Natural prodUCts (COCONUT) is a comprehensive open database that aggregates data from numerous public sources. Originally launched in 2021 [1], COCONUT has undergone a major overhaul with version 2.0 to improve its infrastructure, data quality, and user experience. COCONUT 2.0 [2] contains over 695,000 unique natural product structures compiled from 63 data collections. COCONUT 2.0 provides core information including chemical structures, names/synonyms, source organisms, geographic origins, and literature references. Computed molecular properties and chemical classifications are also included. Key improvements in version 2.0 include a complete redesign of the web interface and backend architecture, enhanced data curation and standardisation processes, new features for community-driven curation and data submission, improved search capabilities—including structure, substructure, and similarity searches—a REST API for programmatic data access, downloadable datasets in multiple formats, and detailed provenance tracking and audit logs. Since COCONUT is an aggregate, issues from its constituent datasets, such as occurrences of synthetic compounds and inaccurate metadata, inevitably appear in the database. By harnessing advanced language models, agentic flows, and community contributions, the team is systematically refining the dataset to eliminate misclassifications and expand genuine natural product entries. With its open licenses, COCONUT remains a robust, evolving resource dedicated to advancing natural product research and its diverse applications.

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P561 nmrXiv – Transforming NMR data archiving, sharing, and reuse

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Machine learning is revolutionizing scientific research by leveraging extensive datasets. A notable example is AlphaFold [1], whose breakthroughs in protein structure prediction have underscored the value of high-quality data from extensive open-access repositories. Initiatives such as NFDI-4Chem [2], part of Germany's National Research Data Infrastructure, are redefining how chemistry research data is managed. These efforts have led to the development of specialized platforms that enhance data sharing and reproducibility. Nuclear Magnetic Resonance (NMR) spectroscopy remains a cornerstone in natural products research and metabolomics, offering essential structural insights for complex molecules. Despite the emergence of several public NMR databases over the past decades, many suffer from incomplete metadata and limited search capabilities [4], while commercial databases—though often more comprehensive—face licensing constraints that restrict their accessibility [5].

To bridge this gap, nmrXiv [3] has been developed as an open, consensus-driven repository and computational platform of published, newly discovered natural products. nmrXiv aims to accelerate coordination and data sharing among researchers by providing a comprehensive platform for managing, sharing, and analyzing both raw and processed NMR spectral data. By integrating raw data, machine-readable metadata, and interactive web-accessible spectra, nmrXiv improves data quality and enhances the user experience. This innovative approach not only streamlines NMR reporting but also catalyzes machine learning applications in spectral prediction and molecular structure elucidation, paving the way for a more collaborative and sustainable research future.

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P562 Emerging nanoformulations of *Plectranthus*-derived abietane diterpenoids for cancer treatment

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Nanoparticles (NPs) play a crucial role in enhancing drug delivery systems by boosting bioavailability and enabling targeted release, particularly when used in combination with natural compounds [1]. This synergy can lead to more efficient treatments with fewer adverse effects. When natural products are encapsulated within NPs, the resulting formulations often demonstrate improved drug loading, controlled release profiles, prolonged circulation time, and reduced toxicity. Among various nanoparticle platforms, metal-based NPs especially gold nanoparticles (**AuNPs**) are particularly attractive due to their straightforward synthesis, stability, and distinct physicochemical properties. These attributes make them well-suited for delivering both drugs and genes, especially in the context of cancer therapy. Abietane diterpenoids, such as 7 α -acetoxy-6 β -hydroxyroyleanone (**Roy**), derived from *Plectranthus* species, have shown cytotoxic activity. However, their clinical potential is hampered by poor aqueous solubility and limited bioavailability. To overcome these challenges, **Roy** and a related esterified compound were incorporated into AuNP-based nanocarriers [2]. These formulations were evaluated based on parameters such as particle size, polydispersity index (PDI), surface charge (zeta potential), and drug encapsulation efficiency. In vitro anticancer studies using breast cancer cell lines (MDA-MB-231, 4T1, and MCF7) indicated that **Roy**-loaded **AuNPs** exhibited significantly stronger cytotoxicity compared to either **Roy** alone or unmodified AuNPs. Furthermore, the **Roy-AuNPs** displayed selective activity against cancer cells while sparing normal human dermal fibroblasts (HDFs), suggesting their potential as targeted anticancer agents.

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P564 Supercritical Fluid Chromatography (SFC) replaces chlorinated solvents for chinese traditional medicine compound purification!

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Dichloromethane (DCM) and other chlorinated solvents are commonly used for chromatography because of their polarity, aprotic structure, and low boiling point. However, these solvents are considered hazardous, with their usage in the United States and elsewhere becoming restricted [1]. There has been much work to replace chlorinated solvents for chromatography, but because none are a drop-in replacement, several combinations of solvent need to be tested using thin layer chromatography plates to find the best combination of solvents. Supercritical fluid chromatography (SFC) is a viable replacement for chromatography using chlorinated solvents. Although carbon dioxide is non-polar, it is polarizable and miscible with alcohols. Method development for SFC is very easy using scouting gradients. Most compounds only need methanol as a co-solvent, making method development easier.

Model compounds that were purified with liquid chromatography with dichloromethane and substituted mobile phases are run with SFC; examples of Chinese traditional Medicine compounds purified with chlorinated solvents are shown with SFC. In all cases, no chlorinated solvents were needed for SFC.

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P565 Remediation of pyrrolizidine alkaloids in plant extracts: a continuous liquid-liquid chromatography approach for red clover

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Red Clover (*Trifolium pratense*) is widely acknowledged in traditional medicine for its therapeutic potential in managing osteoporosis, cardiovascular diseases, arthritis, eczema, asthma, and other conditions. Its beneficial effects are primarily attributed to the rich content of isoflavones, which exhibit estrogenic and antioxidant properties. Pyrrolizidine alkaloids (PAs) are toxic secondary metabolites with hepatotoxic and carcinogenic properties. Red Clover extracts may become contaminated with PA-containing weeds upon harvesting and processing. PA remediation is crucial for safety and therapeutic efficacy; however, efficiently reducing PA levels while preserving isoflavones remains a significant challenge, especially on an industrial scale [1]. Centrifugal Partition Chromatography (CPC) is a liquid-liquid chromatographic technique that separates solutes based on their differential partitioning between two immiscible phases. Compared to batch processing, continuous CPC can enhance productivity and scalability. Continuous operation in CPC is enabled by Multiple Dual Mode (MDM), where periodic shifts between ascending and descending modes alternate the roles of the mobile and stationary phases in each cycle [2]. This study evaluated the effectiveness of CPC in continuous manufacturing mode for the removal of PAs from crude Red Clover extracts while preserving isoflavone content. Additionally, its performance was compared to conventional batch processing. Results demonstrated that alkaloid content was reduced by over an order of magnitude, achieving regulatory compliance (<0.4 ppm), while isoflavone levels remained stable. Continuous mode injection improved alkaloid remediation productivity more than threefold compared to batch processing, without a notable rise in solvent consumption, highlighting the efficiency of the Continuous CPC method (► Table 1).

► Table 1 Comparison of different sample input methods

	Batch	Continuous
Throughput [g input/h]	54	247.5
Injected volume [L/h]	1.2	5.5
Flow rate [mL/min]	200	200
Total Solvent Consumption [L/h]	14.3	18
Output [g/h] Productivity [g/L * h]	44.3	203
Productivity [g/L * h]	3.098	11.278

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P566 Efficacy and bioavailability of rose hip peels extract bioactive compounds for joint health after mimicking human digestive conditions

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Maintaining an active lifestyle is essential at all ages, especially as aging can lead to joint stiffness and reduced flexibility. Natural solutions like rosehip peel extract (Agilirose, patented under WO2009080778) offer support for joint health due to their anti-inflammatory properties [1, 2]. This study investigated the bioavailability of bioactive compounds from rosehip (*Rosa Canina*) extract after simulated gastrointestinal digestion, using a water-based herbal extract (Cynosbati p.e., 70% native/30% maltodextrin). The digestion simulation mimicked the oral cavity, stomach, and small intestine under fasted-state conditions. to assess the release and absorption of active compounds. Subsequently, the resulting digest was permeated through biomimetic membrane inserts to simulate digest absorption, and then collected. In the second part of the experiment, to evaluate the cytotoxicity of the digested contents, experiments were performed using human immortalized fibroblast-like synoviocytes (HS-FL) isolated from healthy human synovial tissue (P10972-IM, Innoprot, Spain) and later on, the absorbed fraction was then applied to cultured synovial cells-typically found in joint tissue – to evaluate effects on inflammation and extracellular matrix components. Results showed a significant reduction in inflammatory markers such as interleukin-6 (IL-6) and matrix metalloproteinase-13 (MMP-13). This physiologically relevant simulation, reflecting how the extract is modified by enzymes and pH during digestion, provides a deeper understanding of the bioactive potential of rosehip compounds in the human body. These findings support and expand upon existing evidence of rosehip extract's anti-inflammatory and joint-protective effects observed in human studies.

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P567 Modulation of Tropomyosin-related Kinase (Trk) signaling by *Ballota nigra* L., *Crataegus oxyacantha* L., *Passiflora incarnata* L. and *Valeriana officinalis* L. in vitro

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We reported earlier the modulation of the neurotrophic activity by the standardized plant extracts of *Ballota nigra* L., *Crataegus oxyacantha* L., *Passiflora incarnata* L. and *Valeriana officinalis* L. alone and in combination [1]. Data were obtained from *in vitro* studies in human neuroblastoma SH-SY5Y cells treated with the plant extracts and Lorezapam (LZ) as reference drug. Neither single plant extracts nor the combination nor LZ reduced cell viability or stimulated TNF-α or IL-1β releases. They induced GABA- and melatonin-releases and increased the gene expression (GE) of the brain derived neurotrophic factor (BDNF). Recently a positive allosteric modulation of neurotrophin/Trk receptors was proposed to influence mood disorders as a potential new therapeutic concept [2]. To investigate the neurotrophin/Trk-signaling by the four plant extracts alone and in combination and LZ as reference.

Based on deep sequencing data obtained from our previous study¹, the GEs involved in the neurotrophin/Trk-signaling pathway (Log2FC, average expression, p-value) were analyzed for all single plant extracts and their combination in three different dosages (2.5, 5, 25 µg/ml) and for LZ. RT-PCR was performed for selected genes involved in neurogenesis/neuroprotection.

Results: At high concentrations all four plant extracts, LZ and the plant combination significantly modulated members of the neurotrophin/Trk signaling pathway with individual profiles, with NTRK2 (TrkB) regulated most prominently. The GE of superoxide dismutase (SOD) was increased concentration-dependently by the four plant extracts, LZ and the plant combination.

Based on GEs all four plant extracts and their combination activate the neurotrophin/Trk-pathway while oxidative stress is reduced.

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P568 Rapid detection of anti-inflammatory compounds: a novel TLC-based enzyme assay

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Arachidonate 5-lipoxygenase (ALOX5) plays a key role in chronic inflammatory diseases and allergic conditions through the production of inflammatory mediators, making it a prime target for the development of anti-inflammatory compounds. Conventional ALOX5 inhibition assays rely mainly on quantification of enzyme products by HPLC or using expensive commercial kits, which are not suitable for screening complex natural extracts. In contrast, TLC-based enzymatic assays, which combine chromatographic separation followed by in situ enzymatic reaction, provide a simple, rapid and cost-effective method for directly visualizing enzyme inhibition, making them ideal to screen complex mixtures [1, 2]. Here, we introduce a simple and cost-effective TLC-based assay to detect rapidly ALOX5 inhibitors in crude extracts using a chromogenic reaction. The assay exploits the oxidative coupling of MBTH and DMAB during enzymatic arachidonate conversion, producing a grey-blue color indicative of lipoxygenase activity [3]. This enables the direct visualization of ALOX5 inhibitors as clear spots on the TLC plate. Validation with known ALOX5 inhibitors such as zileuton confirmed the method's reliability, showing dose-dependent inhibition. Screening of plant extracts demonstrated that the assay allows the detection of potential ALOX5 inhibitors from natural sources. This TLC-based enzyme assay offers therefore a rapid, affordable, and efficient alternative for the preliminary screening of ALOX5 inhibitors, facilitating the discovery of new anti-inflammatory compounds.

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P569 Gaining ultrastructural insight into the chitin layer of the model organism *Saccharomyces cerevisiae* to mediate drug development research

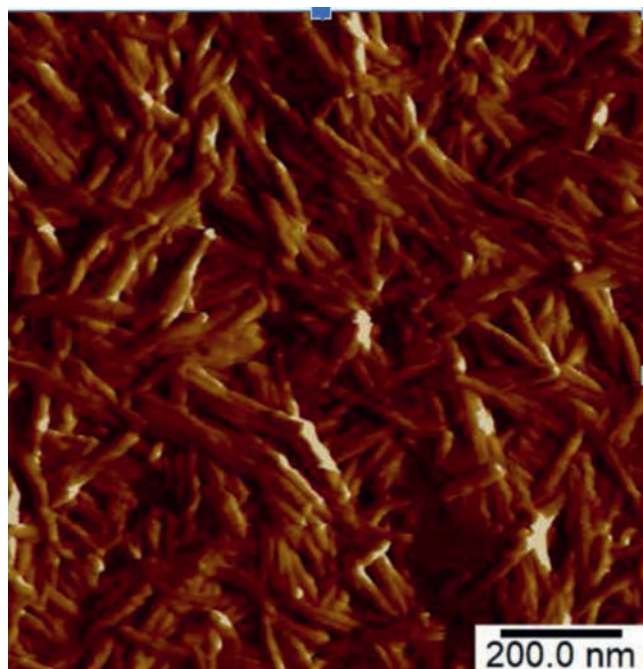
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In the last decades, systemic fungal infections became a rising problem of global public health [1], mainly because resistances of fungal pathogens against common antimycotics like azoles, echinocandins and polyenes have increased strongly [2]. Hence, the development of innovative antimycotics is of specific importance today. Since the unique triple-layered fungal cell wall is required for cell stability and cell division, it is considered a promising target to develop selective and efficient antimycotic drugs [3]. In anti-infective drug research, lead structures frequently lack effectiveness against whole cells [4] because of defense mechanisms exerted by the targeted pathogens (e.g. activity of efflux transporters or limited cell wall permeability).

To identify chitin inhibitors with activity against whole cells, we developed fluorescence and atomic force microscopy (AFM) bioimaging assays allowing to evaluate effects of chitin-synthase inhibitors on the composition and architecture of the cell wall chitin layer on a single cell basis. With the ability to isolate and nanoscopically characterize the chitin layer of the model organism *Saccharomyces cerevisiae* var. *boulardii* by AFM, effects of antifungal drug candidates can be studied in unprecedented detail (► Fig. 1).



► **Fig. 1** Isolated chitin fibrils of *Saccharomyces Cerevisiae* captured with Atomic Force Microscopy

In conclusion, our assays allow closer insight into chitin synthesis inhibition and will prospectively enable the direct assessment of antifungal effects against whole cells. Altogether, these assays could simplify the development of cell wall inhibiting antimycotics.

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P570 Yield, components and antioxidant activities of thyme, oregano, savory essential oils from Türkiye

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Türkiye is an important country in terms of thyme diversity and trade [1]. In this study, a total of seven different thyme, oregano and savory taxa were collected and identified during the flowering period, and their essential oil components and antioxidant activities were investigated. The hydrodistilled essential oils from dried plants were analyzed by GC-MS. The essential oil yields ranged from 0,12 % to 1,55 %. The major components in essential oils of seven taxa were identified as caryophyllene (14.78 %) and germacrene-D (14.74 %) for *O. vulgare*, carvacrol (15.36 %) for *S. cuneifolia*, thymol (37.94 %) and γ -terpinene (21.34 %) for *S. hortensis*, β -terpinyl acetate (12.47 %) and (-)-spatulenol (10.01 %) for *S. wiedemanniana*, caryophyllene (23.28 %) and carvacrol (16.12 %) for *T. leucotrichus*, 1,8-cineole (10.36 %) for *T. longicaulis*, 1,8-cineole (13.80 %) and trans-caryophyllene (11.73 %) for *T. sipyleus*. Carvacrol and thymol compounds representing thyme species were detected together in *S. cuneifolia*, *S. hortensis*, *T. leucotrichus* and *T. longicaulis*, while only carvacrol was detected in *O. vulgare* and *S. wiedemanniana*. These compounds were not recorded in *T. sipyleus*. The ability of the tested essential oils to scavenge DPPH radicals varied between 36.9–88.9 %. Among the standards, ascorbic acid was the highest with 89.2 %, while thymol was the lowest with 6.0 %. The essential oils of all thyme taxa showed higher activity than thymol and butylhydroxytoluene (BHT). *S. hortensis* and *S. cuneifolia* taxa showed higher antioxidant activity than carvacrol standard, but lower activity than ascorbic acid. *S. hortensis*, *S. cuneifolia* and *T. longicaulis* taxa showed stronger activity than trolox standard. The most promising taxa for use in the pharmaceutical and food industries might *S. hortensis*, *S. cuneifolia* and *T. longicaulis*.

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P571 The role of insect pollination in shaping the chemical composition of edible plants: A Comparative Study on multiple fleshy fruit crops

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Pollination plays a fundamental role in shaping the chemical features of edible plants, yet its impact remains largely underexplored compared to genetic and agronomic factors. Understanding how pollination influences phytochemical traits is essential for optimizing crop quality, nutritional value, and sustainability. This study investigates how insect pollination modulates the metabolic profile of blackberries, raspberries, and Solanaceae crops (cherry and date tomatoes, chili peppers) compared to self-pollination.

Using a targeted, multi-matrix analytical approach, this study quantitatively evaluates the content of free sugars in blackberries, raspberries, and tomatoes, phenolic compounds in blackberries and raspberries, capsaicinoids in chilli pepper, and carotenoids in Solanaceae fruits. Optimized extraction protocols combined with high-performance liquid chromatography (HPLC) coupled with UV, RID, FLD, and HRMS detection allow precise identification and quantification of these bioactive and sensory-impacting compounds. A comparative evaluation of pollination systems provides insights into the metabolic pathways most influenced by insect activity and their role in defining fruit quality.

Preliminary analyses were conducted across all studied crops, assessing target analytes relevant to each matrix. These analyses ensured the exclusion of experimental confounding factors such as field-related biases, strengthening the reliability of subsequent comparisons. Cherry tomatoes and blackberries represent the first dataset where a complete analytical workflow has been applied, allowing an initial evaluation of the impact of pollination on metabolic composition.

By unveiling how pollination shapes the phytochemical landscape of key crops, this study bridges a crucial gap in agri-food research, with significant implications for sustainable agriculture, functional food development, and the future of plant-based nutrition.

P572 Flavonoids from *Plukenetia volubilis* with potential anti *Helicobacter pylori* activity

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Helicobacter pylori infection is a significant global health issue, with widespread prevalence linked to conditions such as gastritis, peptic ulcers, gastric cancer, and mucosa-associated lymphoid tissue (MALT) lymphoma. The effectiveness of current treatment protocols is hindered by increasing antibiotic resistance and patient compliance challenges, primarily due to lengthy regimens and side effects. In this context, research into the antimicrobial properties of *Plukenetia volubilis*, commonly known as sacha inchi, represents a promising area due to its rich source of bioactive compounds. However, studies specifically examining its antimicrobial activity against *H. pylori* are limited.

This study investigated the anti-*H. pylori* activity of *P. volubilis* leaf water extract using both in vitro and in silico methods. The chemical composition of the water extract was characterized using High-Performance Liquid Chromatography coupled with Electrospray Ionization and Quadrupole Time-of-Flight Mass Spectrometry (HPLC-ESI-QTOF-MS-MS). This analysis identified several flavonoids, including astragalin, as well as various phenolic compounds. High-speed counter-current chromatography (HSCCC) was then employed to fractionate the ethyl acetate partition obtained from the water extract of the leaves.

Notably, the confirmation of flavonoids derived from kaempferol and the isolation of compounds such as astragalin, kaempferol-3,7-di-O-glucoside, and kaempferol-3-O-sophoroside represent significant advancements in our understanding of the bioactive compounds in *P. volubilis*. The water infusion from *P. volubilis*, its butanol extract, and the isolated astragalin exhibited antibacte-

rial activity against *H. pylori* strain J99 and two clinical isolates, further emphasizing their potential (e.g., minimum inhibitory concentrations of 0.53, 0.59, and 0.49 µg/mL, respectively, against a clarithromycin-resistant clinical isolate). *In silico* analysis through molecular docking revealed that astragalin could interact with potential protein targets associated with *H. pylori*, adding further depth to our findings. These results highlight the bacteriostatic activity of *P. volubilis* and astragalin against *H. pylori*; however, more research is needed to fully explore and utilize the potential of these bioactive compounds in treating *H. pylori* infections.

P573 A fresh plant-based approach to sleep and stress modulation: Analytical and pharmacological insights into *Eschscholzia californica* herba soft extract

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Eschscholzia californica (California poppy) has a longstanding history of traditional use for relieving mild mental stress and for promoting sleep [1]. We report ongoing analytical and pharmacological research on a novel hydroethanolic soft extract derived from fresh aerial parts of *E. californica*. Multibatch comparisons across different harvest years and regions using various analytical techniques (HPTLC, HPLC, ¹H NMR) revealed consistent phytochemical fingerprints with preserved levels of key alkaloids and flavonoids. A comparison between dried herbal preparations and the fresh plant soft extract is shown and discussed with respect to the identity and quantity of the major natural products.

To explore potential mechanisms of action in relation to analytically defined sample characteristics, a receptor binding screen was conducted across 53

CNS-relevant targets. The analysis reveals high-affinity interactions with serotonergic (5-HT_{1B}, 5-HT₆, 5-HT₇), dopaminergic (D₁, D₃, D₅), and adrenergic (α_{1A}, α_{2C}) receptors, while no relevant binding is observed at melatonergic (MT₁), adenosinergic (A₁, A_{2A}, A_{2B}), or orexinergic receptors. These findings are currently being corroborated using additional extract batches and functional receptor assays.

Preliminary results suggest a non-GABAergic, multi-target mode of action supporting stress resilience, reduced rumination, and facilitated sleep onset, in line with preliminary findings [2, 3]. This pharmacological profile complements clinical observations from past studies with *Eschscholzia* preparations showing improvements in sleep latency, quality, and anxiety reduction [4]. Combined with robust analytical control, the extract offers a promising phytopharmaceutical strategy for stress-related sleep disturbances.

Conflict of Interest P.K, U.B & R.W. are employees of the sponsor of the studies, which was A.Vogel AG. J. M. L-R received honoraria from the sponsor of the studies.

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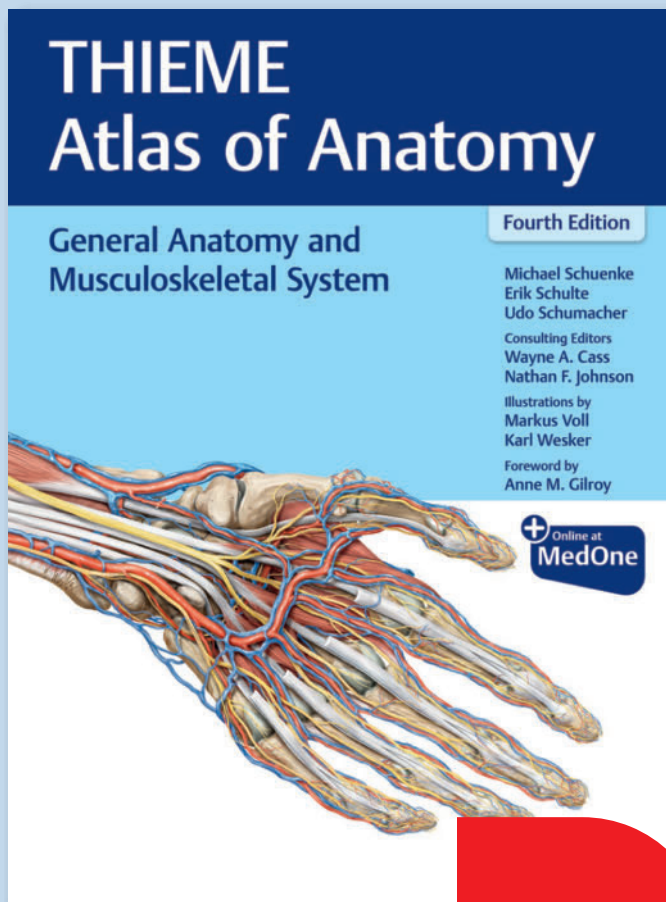
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