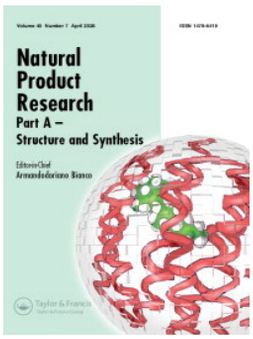

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Chemical constituents and antibacterial activities of Cameroonian dark brown propolis against potential biofilm-forming bacteria

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ABSTRACT

Propolis is a resinous material collected by different bee species from various plant exudates and used to seal holes in honeycombs, smoothen the internal walls, embalm intruders, improve health and prevent diseases. From its *n*-hexane extract, eight compounds were isolated and characterised as: mangiferonic acid (**1**); 1-hydroxymangiferonic acid (**2**), new natural product; mangiferolic acid(**3**); 27-hydroxymangiferolic acid (**4**), reported here for the first time as propolis constituent; 27-hydroxymangiferonic acid (**5**); *α*-amyrin (**6**); *β*-amyrin (**7**) and lupeol (**8**). The chemical structures of the isolated compounds were elucidated using spectroscopic methods, such as 1D and 2D-NMR, mass spectrometry and comparison with previous published reports. Compounds **6-8** and *n*-hexane extract were tested against Gram-negative and Gram-positive bacteria strains using agar disc diffusion and macrodilution techniques. Interestingly, *n*-hexane extract and compounds **6-8** had good inhibitory activities against Methicillin Resistant *Staphylococcus aureus* (MRSA) and the clinical *Klebsiella pneumoniae* isolates. The biological effects of *n*-hexane extract and its fraction against *K. pneumoniae* 12CM and MRSA revealed in the present study suggest that the Cameroonian dark brown propolis could be a potential alternative management of biofilms on medical devices and respiratory skin or infections.

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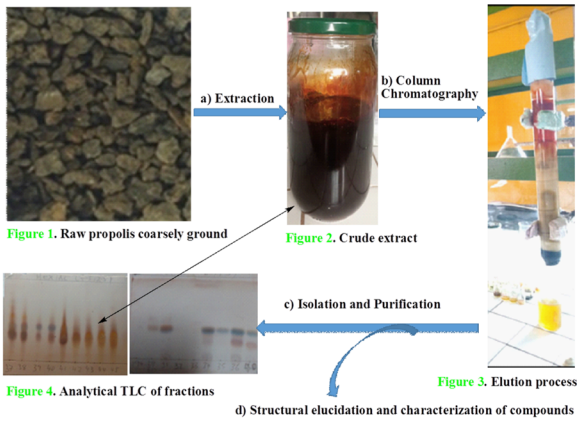

Figure 1. Raw propolis coarsely ground

Figure 2. Crude extract

Figure 3. Elution process

Figure 4. Analytical TLC of fractions

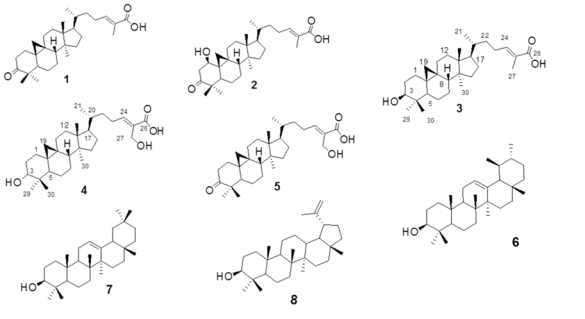

Figure 5. Structure of the isolated compounds

1. Introduction

Propolis is a resinous material collected by different bee species (Abd Jalil et al. 2017) from various plant exudates across the globe and used to seal holes in honeycombs, smoothen the internal walls, and protect the entrance against intruders (Sulaiman et al. 2011; Sakava et al. 2014). Based on colour, propolis varies from dark-brown, dark-yellow, greenish-brown, to red, due to its age and nearby plant sources (Devequi-Nunes et al. 2018). This bee glue has been used extensively in cosmetics, the beverage industry, and foods to improve health and prevent diseases such as inflammation, heart disease, diabetes, microbial infections, and even cancer (Sulaiman et al. 2012; Silva-Carvalho et al. 2015; Tran et al. 2020; Shabani et al. 2021; Alanazi 2022). It is produced by honeybees (*Apis mellifera*) to sterilise the hive environment, ensuring a healthy living condition for the bee colony due to its antibiotic properties (Bankova et al. 2018). Unlike the propolis generated by honey bees, geopropolis is a distinctive kind of propolis produced by stingless bees (*Meliponini*) (Chuttong et al. 2023). Stingless bees incorporates wax and soil into the composition of geopropolis, resulting in its unique attributes (Chuttong et al. 2023). In addition to these biological potentials, propolis and its components have recently been widely examined and scrutinised for over 400 chemical compounds including several polyphenols, flavonoids, chromones, phenolic acid and their esters, phenolic aldehydes and ketones, terpenes,

sterols, vitamins, amino acids, waxy acids, sugars, etc (Marcucci 1995; Lotfy et al. 2006; Trusheva et al. 2007**;** Kumar et al. 2009; Jaqueline et al. 2015; Jabir et al. 2018; Mahamat et al., 2020). The majority of the biological effects of propolis could be attributed to its chemical constituents (Fitzpatrick et al. 2019), but not all compounds have biological activity (Sakava et al. 2021; Alorfi 2022). However, most of the studies carried out have not been aimed at determining a complete chemical composition, but were limited to some components of interest, particularly the flavonoids (Popova et al. 2005; Silva et al. 2008, Mello et al. 2010). Besides the chemical complexity, propolis can vary in its biological properties, which significantly effects propolis quality. Other factors such as botanical origins, bee species, and the extraction process, have a direct impact on the quality of the final propolis extract (Chuttong et al. 2023). Therefore, vigorous approaches comprising various chromatographic and spectroscopic techniques are warranted to standardise and isolate pure propolis components and to test their efficacy using clinical trials. Propolis from honeybees is well-known for its antitumoral, antioxidant and antimicrobial activities particularly against biofilm-forming bacteria (Choudhari et al. 2012). It is thus, a natural product with therapeutical potential in demand. Biofilm producing bacteria commonly isolated from medical devices may originate from the skin of patients or health-care workers, intravenous infusions, or other sources in the environment. This group of bacteria are known to exhibit greater resistance to antibiotic agents (Donlan 2021). As a result, they constitute a serious threat to public health and a global burden.

The present investigation reports the extraction, isolation, structure elucidation and antimicrobial properties of a honeybee propolis sample collected from Mezam division in the north-west region of Cameroon. The outcomes of this study will increase knowledge of propolis as a potential nutraceutical agent with therapeutic benefits against bacterial infections particularly caused by biofilm formers such as *Escherichia coli*, *Salmonella* spp, *Klebsiella pneumoniae, Staphylococccus aureus, Pseudomonas aeruginosa* and *Enterococcus faecalis*.

2. Results and discussion

2.1. Structure elucidation

Compound **1** was isolated as an amorphous white powder from the mixture of *n*-hexane-ethyl acetate (87.5-12.5%). The ES^+ mass spectrum showed a pseudo-molecular ion peak $[M + Na + NH_3]^+$ at *m/z* 494.8 (calc. 494.3610 for $C_{30}H_{49}NNaO_3^+$). The ^1H NMR spectral data ($CDCl_3$; 600 MHz; Table S2) of compound **1** exhibited characteristic peaks, some of which were similar to those of cycloartenol. It displayed a set of AB doublets at δ_H 0.58 and 0.79 characteristics of a cyclopropane moiety methylene protons H*α*-19 and H*β*-19, respectively; it showed proton signals at δ_H 1.92 (1H, m) and 2.02 (1H, m) corresponding to H*α*-23 and H*β*-23; at δ_H 6.90 (1H, br t) characteristics of an olefinic proton (H-24); at δ_H 1.00 (3H-18, s); 0.91 (3H-21, d); 1.84 (3H-27, s); 0.91 (3H-28, s); 1.05 (3H-29, s) and 1.10 (3H-30, s) corresponding to six angular methyl groups.[13]C NMR spectral data ($CDCl_3$; 600 MHz; Table S1) exhibited carbon signals at δ_C 145.7 and 126.6. The DEPT 135 experiment characterised the carbon atoms in compound 1, identifying 6 methyl groups at δ_C 18.1 (C-18), 18.3 (C-21), 11.9 (C-27), 19.3 (C-28),

22.2 (C-29), and 20.8 (C-30); 11 methylene groups, including a cyclopropyl methylene carbon at δ_C 29.6 (C-19); 5 methine carbons; and 8 non-protonated carbons, one of which is at δ_C 172.8 (C-26) (Table S1). HSQC and ^{1}H-^{1}H COSY experiments confirmed the presence of an olefinic methine at δ_H/δ_C: 6.90/145.7 and a cyclopropyl methylene group at δ_H/δ_C 0.79 and 0.58/29.6. Literature surveys on the constituents of Cameroonian propolis (Talla et al. 2017; Sakava et al. 2021; Tamfu et al. 2022), and Indonesian propolis (Pujirahayu et al. 2019), revealed cycloartane-type triterpenoids as frequently encountered constituents. All the above evidence confirmed that compound **1** was identified as mangiferonic acid (Figure 1), previously isolated from ether-soluble fraction of the *Tetragonula sapiens* bee propolis and *Mangifera indica* resin (Escobedo-Martínez et al. 2012; Pujirahayu et al. 2019).

Compounds **2** and **3** were obtained as an inseparable mixture (almost 1:1 ratio) of white amorphous powder from *n*-hexane-EtOAc (17%) and gave positive results for the *Liebermann-Burchard* reaction. The ES$^+$ mass spectrum (Figure S11) showed a pseudo-molecular ion peak $[M_2+M_3 + H_2O + 2Na + NH_4]^{3+}$ at *m/z* 1008.1 (calc. 1008.7244 for $C_{60}H_{100}NNa_2O_8^{3+}$); a base ion peak $[M_3+H+2NH_4]^{3+}$ at *m/z* 493.9 (calc. 493.4369 for $C_{30}H_{57}N_2O_3^{3+}$); important fragments were also observed: $[M_2+N_a]^+$ at *m/z* 493.6 (calc. 493.3294 for $C_{30}H_{46}N_aO_4^+$), $[M_2+H]^+$ at *m/z* 471.8 (calc. 471.3474 for $C_{30}H_{47}O_4^+$) from which the molecular formula was deduced as $C_{30}H_{46}O_4$ (**2**), indicating eight degrees of unsaturation and $C_{30}H_{48}O_3$ (**3**) with seven degrees of unsaturation. The NMR spectra ($CDCl_3$, 600 MHz) of compounds **2** and **3** revealed the nature of the cycloartane triterpenoidal skeleton, showing the same HSQC and HMBC spectral patterns except for the difference in the chemical shifts around C-1, C-3, C-24(C-25) and C-26. Compound **3** with OH group at C-3 expressed δ_H about 3.30 ppm and δ_C 78.9. The ^{1}H NMR

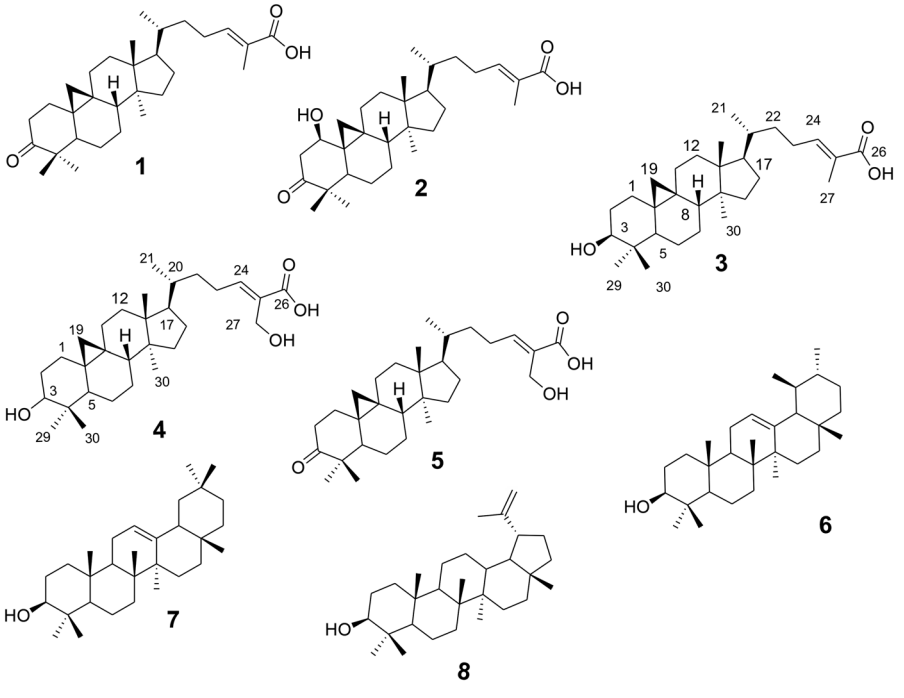

Figure 1. Structure of the isolated compounds.

spectral data of compound **3** (Figure S12; Table S2) showed methyl group as a broad and large singlet at δ_H 1.88 attributed to H-27. The triplet at δ_H 6.93 was attributed to the olefinic proton attached to C-24 of compounds **2** and **3**. DEPT 135 and HSQC spectra confirmed the presence of an additional oxymethine carbon at δ_H/δ_C (3.51 (t)/77.22) . The ^{1}H NMR spectrum (Figure S12) showed a set of AB doublets at δ_H 0.38 and 0.55 for compound **2**, δ_H 0.38 and 0.59 (1H each) for compound **3**, which are characteristics of a 9,19-cycloartane methylene protons H*α*-19 and H*β*-19, respectively. The ^{13}C NMR spectrum (Figure S13) indicated carbon signals at δ_C 171.7 and 171.6; δ_C 145.7 and 145.8; δ_C 126.3 and 126.4 which were assigned to C-26, C-24 and C-25 of compounds **2** and **3**, respectively. These ^{1}H and ^{13}C NMR spectral data (Table S1 and Table S2) closely resembled to those of compound **1** and mangiferolic acid (Pujirahayu et al. 2019). Unfortunately, we observed the absence of the carbon signal at about δ_C 33.4 (C-1) and the appearance of the oxymethine carbon signal at δ_C 77.2.^{1}H–^{1}H COSY experiment (Figure S15) confirmed the presence of an oxymethine function (proton signal at δ_H 3.51 (t, H-1) correlating with the methylene protons at δ_H 2.31 (H-2*α*, d) and 2.71 (H-2*β*, d) for compound **2**. From the previous discussion, along with the HMBC spectrum's investigation and the published data (Pujirahayu et al. 2019; Tamfu et al. 2022), compound **2** was concluded to be 1-hydroxymangiferonic acid, a new natural product and compound **3** was identified as mangiferolic acid (Figure 1).

Compound **4** was isolated as an amorphous white powder in the fraction of *n*-hexane-EtOAc (20%) and responded positively to the Liebermann-Burchard reaction. The ES$^+$ 5.75e4 mass spectrum (Figure S7) showed a *pseudo*-molecular ion peak $[M+15+H_2O+Na+2H]^{3+}$ at *m/z* 529.2 (calc. 529.3869 for $C_{31}H_{54}NaO_5^{3+}$), a base ion peak $[M+15+Na]^+$ at *m/z* 509.9 (calc. 509.3607 for $C_{31}H_{50}NaO_4^+$), another important ion fragment $[M+Na]^+$ appears at *m/z* 495.9 (calc. 495.3450 for $C_{30}H_{48}NaO_4^+$) from which the molecular formula, $C_{30}H_{48}O_4$ was deduced, having seven degrees of unsaturation. The ^{1}H NMR spectrum ($CDCl_3$, 600 MHz, Figure S8; Table S2) of compound **4** showed proton signals at δ_H 6.94 (1H, t) characteristic of a conjugated trisubstituted olefin proton (H-24); δ_H 4.30 (2H, brs) attributed to oxymethylene protons (2H-27); δ_H 3.21 (1H, dd) assigned to oxymethine proton H-3; δ_H 2.29 and 2.15 (2H, m); δ_H 0.49 (1H, d) and 0.27 (1H, d) characteristics of a cyclopropyl methylene protons H*α*-19 and H*β*-19, respectively. Finally, signals of five methyl protons at δ_H 0.82 (3H-18, s); 0.85 (3H-21, d); 0.74 (3H-28, s); 0.90 (3H-29, s) and 1.04 (3H-30, s). ^{13}C NMR spectrum ($CDCl_3$; 125.8 MHz; Figure S9; Table S1) of compound **4** exhibited signals at: δ_C 18.0 (C-18), 18.1 (C-21), 19.3 (C-28), 22.2 (C-29) and 20.8 (C-30) attributed to the five angular methyl groups; δ_C 29.6 assigned to C-19, δ_C 57.1 granted to C-27; δ_C 78.9 corresponding to C-3; δ_C 149.1 (C-24) and δ_C 129.6 (C-25) allocated to two olefinic carbons; and δ_C 170.8 (C-26) attributed to an *α*,*β*-unsaturated carbonyl of the carboxylic acid carbon. These NMR spectral data (Table S1 and Table S2) closely resembled to those of methyl-3*β*,27-dihydroxymangiferolic acid (Talla et al. 2017). From all above, compound **4** was identified as 27-hydroxymangiferolic acid, previously isolated from the stem bark of *M. indica* (Escobedo-Martínez et al. 2012).

Compound **5** was isolated as a white amorphous powder in *n*-hexane-EtOAc (85:15). It reacted positively to the Liebermann-Burchard test. Its ES$^+$ mass spectrum (Figure S1) showed a *pseudo*-molecular ion base peak $[M+15+Na+NH_4]^{2+}$ at *m/z* 525.7 (calc.

525.3794 for $C_{31}H_{52}NNaO_4^{2+}$), other important fragments $[M+15+Na]^+$ were observed at *m/z* 507.2 (calc. 507.3450 for $C_{31}H_{48}NaO_4^+$) and $[M+Na+H]^{2+}$ at *m/z* 494.0 (calc. 494.3372 for $C_{30}H_{47}NaO_4^{2+}$). Its molecular formula was deduced to be $C_{30}H_{46}O_4$, indicating 8 degrees of unsaturation. The ^{1}HNMR spectrum (Figure S2A,B) of compound **5** showed a triplet at δ_H 7.05, two cyclopropane proton signals at δ_H 0.82 (d, H-19*α*) and 0.61 (d, H-19*β*), a methyl doublet at 0.95 (H-21), and a proton signal at 4.39 (br s, 2H-27). This ^{1}H NMR spectrum was identical to that of compound **4**, except for the presence of a signal at δ_H 3.21 (dd). The ^{13}C NMR spectrum (Figure S3A,B) showed carbon signals at: δ_C 216.5 (C-3); δ_C 170.8 (C-26); δ_C 149.1 (C-24) and 129.6 (C-25); δ_C 57.2 (C-27), a cyclopropyl methylene carbon signal at δ_C 29.5 (C-19) and five angular methyl carbon signals at: δ_C 18.0 (C-18), 18.1 (C-21), 19.3 (C-28), 22.2 (C-29) and 20.8 (C-30). The HSQC spectrum showed directly the protons bonded to carbons, while the ^{1}H-^{1}H COSY spectrum identified clearly the neighbouring protons connectivity. Finally, HMBC spectrum indicated the correlations between the 2H-27 with C-25 and C-26, while other correlations from H-24 confirmed C-26, C-27, and C-22. Correlations from H-28 and H-29 identified C-3, C-4 and C-5. This ^{13}C NMR spectrum was also identical to that of compound **4**, except for the absence of an oxymethine carbon at δ_C 78.9 and the presence of a ketone carbonyl carbon at δ_C 216.5. This supports the replacement of the hydroxyl group at C-3 with a ketone function. All the above 1D and 2D NMR spectra combined with the published data (Alanazi 2022; Tamfu et al. 2022) identified compound **5** as 27-hydroxymangiferonic acid.

Compounds (mixture, **6-8**) were obtained from the purification column chromatography as white amorphous powder in *n*-hexane/ethyl acetate (95/5) . It reacted positively to the Liebermann-Burchard reaction. In the ^{1}H-NMR spectrum (Figure S17; Table S2), two proton signals were observed at δ_H 5.14 (t), and 5.04 (t), attributed to H-12 of the ursan−12-ene- and olean-12-ene-type triterpenoids. The same ^{1}H-NMR spectrum showed a pair of broad singlets at δ_H 4.62 and 4.49 assigned to the vinylic protons attached to C-29 position of the lup-20(29)-ene-type triterpenoids. Three doublets were observed at δ_H 3.18, 3.14 and 3.04, attributed to three oximethine protons attached to C-3 position each of the pentacyclic triterpenoids. The presence of a singlet at δ_H 1.61 confirmed the attachment of methyl group to the olefinic carbon (C-20) of the lup-20(29)-ene series. The singlet and doublet between 1.09-0.72 were assigned to the angular methyl protons for the pentacyclic triterpenoids. In the ^{13}C-NMR spectrum (Figure S18; Table S1), two carbon signals were observed at δ_C 124.4/139.6 corresponding to the typical resonance values of C-12 and C-13 positions respectively for ursane-type triterpene (Hernández-Vázquez et al. 2012). The characteristic signals at δ_C 59.1 (C-18), 39.7 (C-19), 39.6 (C-20), 31.3 (C-21) and 41.5 (C-22) were attributed to the carbons in the E ring, which in turn were very similar to those of *α*-amyrin (Hernández-Vázquez et al. 2012). This spectrum also exhibited carbon signals at δ_C 121.7/145.2 corresponding to the typical resonance values of C-12 and C-13 positions respectively for an oleane-type triterpene (Hernández-Vázquez et al. 2012). The carbon signals at δ_C 47.3 (C-18), 46.8 (C-19), 31.1 (C-20), 34.7 (C-21) and 37.1 (C-22) were assigned to the E ring, which in turn were very similar to those of *β*-amyrin (Hernández-Vázquez et al. 2012). Two additional olefinic carbon signals were observed at δ_C 109.3/151.0, assigned to the resonance values of C-29/C-20 positions respectively for a lupane-type triterpene (Rosandy et al. 2021). The carbon signals at δ_C 48.3 (C-18), 48.0 (C-19),29.8 (C-21) and 40.0 (C-22) were

attributed to the E ring, resembling those of lupeol. Three oxymethine signals were observed at δ_C 79.06, 79.04, and 79.01 ppm attributed to C-3β-OH position on each of the pentacyclic triterpenoids. All the above spectral data led to the elucidation of our isolated compound as a mixture of α-amyrin (**6**), β-amyrin (**7**) and lupeol (**8**).

It is evident that propolis varies in chemical composition, even in the same geographical location. Triterpenoids obtained here in Bambui propolis n-hexane extract were similar to previous investigations on propolis from Ngaoundal locality of Cameroon, Indonesia, the United Kingdom and philippine (Sakava et al. 2021, 2014; Alanazi 2022). All cycloartane-type triterpenes in our propolis except 1-hydroxymangiferonic acid (**2**) are also present in *M. indica* resin as its most common components (Anjaneyulu et al. 1999; Pujirahayu et al. 2019). 1-ydroxymangiferonic acid (**2**), is a new natural product; 27-hydroxymangiferolic acid (**4**) is the first cycloartane-type triterpenoids found in Cameroonian propolis. The cycloartane-type triterpenes in Bambui propolis were also found in the other Cameroonian propolis from different localities such as Ngaoundal (Adamawa region), Foumban (West region), Babanki village, Oku and Nguabum-Konene (North West region). Some propolis samples were also reported to contain cycloartane-type triterpene compounds: Myanmar (Li et al. 2009; Pujirahayu et al. 2019) (*Apis mellifera*) propolis and Vietnam *Trigona* propolis (Sun et al. 2015), Malaysian propolis (*Trigona itama*) (Woźniak et al. 2019) and Brazilian propolis (Miguel et al. 2010**).**

It is interesting that, although the botanical origin of propolis from Myanmar (Li et al. 2009; Pujirahayu et al. 2019), Vietnam (Nguyen et al. 2017; Pujirahayu et al. 2019), Malaysia (Zayyanu and Mohamed 2015; Pujirahayu et al. 2019), and Brazil (Freitas et al. 2008; Pujirahayu et al. 2019) is the same plant, *M. indica*, the cycloartane-type triterpenes contained in that propolis are not the same. This difference may be due to the *M. indica* variety which is not the same in each region where the propolis samples originate. This difference may be due to the behaviour of bees in collecting resins in colonies, species and varieties of plants, as well as differences in the parts and amount of resin taken. The composition of the secondary metabolites contained in each part of the plant such as shoots, leaves, branches, and stems is not the same even in one plant/tree. Bees can collect resin from the bud exudates or the sap that comes out of the wound on a branch or tree trunk. The differences found between propolis samples from various regions are mainly due to differences in the flora and less to the species of the bees. Several plants around the hive, such as *M. indica* (mango), *Anacardium occidentale*, *Artocarpus cempedan*, *Euphorbia milii*, *Euphorbia pulcherima*, *Euphorbia dendroides*, *Orthosiphon stamineus*, *Boswellia sacra*, *Boswellia neglecta*, *Melipona beecheii*, *Ficus exasperate*, *Byrosonima fagifolia*, *Byrosonima crassifolia*, *Lavandula officinalis*, *Pachysandra terminalis*, *Melandrium firmum*, *Tydemania expeditionis* and some flowering plants could also be visited by honeybees.

The *n*-hexane crude extract and mixture of **6**, **7** and **8** were tested for their antibacterial activities against some bacteria strains.

2.2. Antimicrobial activity

The microorganisms and the control antibiotics were chosen in this study for different reasons: *E. coli* ATCC 25922, *Salmonella* spp 2008365501 are among the major agents

of foodborne diseases while *K. pneumoniae* ATCC 700603 and the clinical *K. pneumoniae* 12 CM are important causes of bacterial respiratory infections. *S. aureus* has been involved in skin infections and food poisoning. Finally, *P. aeruginosa* ATCC 7110 and *E. faecalis* ATCC 51299 are among the major cause of nosocomial infections with great virulence and antibiotic resistance potentials. Additionally, all these organisms are biofilm producing bacteria commonly isolated from medical devices that constitute the WHO priority list of antibiotic-resistant pathogens (WHO 2017). On the other hand, the selected control antibiotics are either critically (Amikacin, Ceftazidime, Ciprofloxacin and Meropenem) or highly important drugs (Chloramphenicol) (WHO 2019).

Generally, the propolis extract and its fraction in this study exhibited differential antimicrobial effects on Gram-positive bacteria as well as on Gram-negative ones (Tables 1 and 2). Except methicillin resistant *S. aureus* (MRSA), the selected Gram-positive bacteria in this study seemed to be less susceptible to the hexanic extracts compared to the Gram-negative organisms. The present study contradicts findings of several previous research works that reported wider antibacterial activity of propolis on Gram-positive bacteria than on Gram-negative ones (Przybyłek and Karpiński 2019; Almuhayawi 2020; Chuttong et al. 2023). Similarly, Béji-Srairi and team-mates surprisingly reported strong antibacterial activity of Tunisian propolis on Gram-negative bacteria (Béji-Srairi et al. 2020). These differences may be due to the fact that the antibacterial potential of propolis varies considerably from one bacterial strain to another, but also depends on the propolis sample used (Almuhayawi 2020). The

Table 1. Antimicrobial activity screening of the propolis *n*-hexane extract, fraction (*α*-amyrin (**6**), *β*-amyrin (**7**) and lupeol (**8**)) and WHO priority antibiotics (diameter of zone of inhibition- mm) against Gram-positive bacteria.

Tested samples	*S. aureus* ATCC 7625	*S. aureus* MRSA	*E. faecalis* ATCC 51299
α-amyrin, β-amyrin and lupeol	R (≤2)	17	R (10)
n-hexane extract	R (≤2)	R (≤2)	R (≤2)
MEM-10	40	32	R (≤2)
CIP-5	28	40	R (≤2)
AK-30	21.5	20	R (≤2)
CAZ-30	21	R (15)	R (≤2)
C-30	28	24	R (≤2)

AK: Amikacin; C: Chloramphenicol; CAZ : Ceftazidime; CIP: Ciprofloxacin; MEM: Meropenem; I : intermediate; R: resistant.

Table 2. Antimicrobial activity screening of propolis the *n*-hexane extract, fraction (*α*-amyrin (**6**), *β*-amyrin (**7**) and lupeol (**8**)) and WHO priority antibiotics (diameter of zone of inhibition- mm) against Gram-negative bacteria.

Tested samples	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 7110	*Salmonella* spp 200836550	*K. pneumoniae* ATCC 700603	*K. pneumoniae* 12 CM
Fraction	15	R(10)	17	R (≤2)	R (≤2)
n-hexane extract	17	20	R (≤2)	R (≤2)	16
MEM-10	30	34	30	33	I (27.5)
CIP-5	35	34	30	I (23)	R (14)
AK-30	20	30	21	25	R (15)
CAZ-30	25	R (22)	21	R (8.5)	R (≤6)
C-30	30	R (7)	25	R (10)	R (21)

AK: Amikacin; C: Chloramphenicol; CAZ : Ceftazidime; CIP: Ciprofloxacin; MEM: Meropenem; I : intermediate; R: resistant.

chemical composition and the mode of action of the active ingredients of propolis underlines the differential activities of propolis on Gram-positive and Gram-negative microbes. Based on documented literature, the double cytoplasmic membrane of Gram-negative bacteria embedding some enzymatic activities make them less sensitive to propolis antimicrobial agents (Ristivojević et al. 2016; Balderas-Cordero et al. 2023; Chuttong et al. 2023). Otherwise, the cell walls of Gram-negative bacteria is poor permeable to propolis active components than those of Gram-positive organisms (Rosandy et al. 2021).

Interestingly, the propolis fraction (mixture of *α*-amyrin, *β*-amyrin and lupeol) had a good inhibitory action against both MRSA and *E. faecalis* with MIC of 12.5 µg/mL (Table 3). MRSA is one of the leading causes of community and hospital-acquired infections and is commonly associated with significant economic burden resulting from high morbidity, and mortality. The anti-staphylococcal activity of propolis is not uncommon as it is extensively documented in the literature (Popova et al. 2011; Choi et al. 2018; Gajdács 2019; Kharsany et al. 2019). Furthermore, *E. faecalis* which, is known to be responsible of community-acquired endocardiris and urinary tract infection (CDC 2013) registered a high antimicrobial activity against the propolis fraction. Curiously, the same *E. Faecalis* isolate showed resistance to all the five WHO priority antibiotics selected in this study. While the *n*-hexane crude extract was inactive against Gram-positive bacteria, paradoxically, it recorded a relatively wider inhibitory effects against Gram negative organisms (active against *E. coli* ATCC 25922, *P. aeruginosa* ATCC 7110, *K. pneumoniae* 12 CM) compared to its fraction (active against *E. coli* ATCC 25922, *Salmonella* spp 200836550). The differential activities of the hexanic extract and its fraction in this study may be due to a variation in their chemical composition. Moreover, the antibacterial activities of the fraction may be linked to the synergistic actions of the three pentacyclic triterpenes *α*, *β*-amyrins and lupeol known to show antimicrobial properties (Vázquez et al. 2012; Rosandy et al. 2021). Though in a Korean study, olean-27-carboxylic acid, a derivative of *β*-amyrin exhibited potent antibacterial activity against strains of MRSA, the present study did not establish the anti-staphylococcal activity of the fraction to the individual biological effect of *β*-amyrin (Vázquez et al. 2012).

Though the propolis *n*-hexane extract and its fraction exhibited a relatively limited antimicrobial effect compared to the five WHO priority antibiotics, their combined individual antibacterial effects against biofim-producers (MRSA, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 7110, *K. pneumoniae* 12 CM and *Salmonella* spp 200836550) underline their pharmacological potential as anti-biofilm agents. This explains reason for its

Table 3. Minimum inhibitory concentrations (µg/mL) of the *n*-hexane extract and fraction (*α*-amyrin (**6**), *β*-amyrin (**7**) and lupeol (**8**)).

Propolis	*E. coli* ATCC 25922	*Salmonella* spp 2008365501	*K. pneumoniae* ATCC 700603	*K. pneumoniae* 12 CM	*Ps. aeruginosa* ATCC 7110	*S. aureus* ATCC 7625	*S. aureus* MRSA	*E. faecalis* ATCC 51299
Fraction	250	62.5	–	–	125	–	125	125
n-hexane extract	–	–	–	15.625	12.5	–	–	–

-: MIC not done.

recommendation as a spray on medical devices to prevent their contamination by biofilm-forming bacteria in a previous study (El-Guendouz et al. 2018).

The antibacterial activities of the *α*-amyrin, *β*-amyrin and lupeol mixture against methicillin-resistant *S. aureus* could encourage its use as ointment for the management of skin infections or as a complementary to drugs including linezolid for the treatment of hospital acquired infections. This goes in line with previous studies that highlighted the use of propolis extract as a healing medicine for the management of skin infections and wounds (Kuropatnicki et al. 2013; Almuhayawi 2020; Kubat et al. 2021). Its application in food safety should also be explored in relation to the inhibitory effect of the fraction against *Salmonella* species. Probably, its therapeutic use for the treatment of bacterial gastrointestinal disorders may be due to its antisalmonella property (Silva et al. 2008). Additionally, the inhibitory effects of the hexanic extract against the clinical *K. pneumoniae* 12 CM, a powerful causative agent of bacterial respiratory infections may encourage its use as a complementary therapy during COVID-19 co-infections (Salatino 2022). In perspective, further studies should be envisaged to evaluate the anti-biofilm and wound healing properties of the present propolis extracts.

3. Experimental

3.1. General experimental procedures (see supplement)

3.1.1. Propolis material

Raw propolis was harvested from several beehives by scraping method in July 2018 from Bambui village (Tubah subdivision, Mezam division) and was supplied to us by the Bee farmers Honey Co-operative Union (HONCO HONEY at Mile 2 Nkwen-Bamenda, North-West region of Cameroon).

3.2. Extraction and isolation (see supplement)

3.2.3. NMR data of compound 2

1-hydroxymangiferonic acid (2): white amorphous powder in *n*-hexane-EtOAc (83:17) %; ES^+MS (Figure S11): *m/z* 471.8 $[M_2+H]^+$, (calculated molecular weight: 471.3474 for $C_{30}H_{46}O_4^+$); ^1H NMR ($CDCl_3$, Figure S12): δ_H 3.51 (t, 7.2, H-1), 2.49 (d, 7.9;, H-2*α*) & 2.24 (d, 4.0, H-2*β*), 1.69 (d, 7.1, H-5), 1.53 (m, H-6*α*) & 0.94 (m, H-6*β*), 1.34 (m, H-7*α*) & 1.14 (m, H-7*β*), 1.50-1.57 (m, H-8), 1.94 (m, H-11*α*) & 1.15 (H-11*β*), 1.64 (m, 2H-12), 1.33-1.37 (m, 2H-15), 1.94-1.98 (m, 2H-16), 1.64-1.69 (m, 2H-17), 1.00 (brs, 3H-18), 0.38 (d, 4.2; H-19*α*) & 0.55 (d, 4.2; H-19*β*), 1.45-1.47 (m, H-20), 0.92 (d, 6.6; 3H-21), 1.58 (m, H-22*α*) & 1.20 m (m, H-22*β*), 2.04 (m, 2H-23), 6.93 (t, 7.3; H-24), 1.88 (brs, 3H-27), 0.93 (s, 3H-28), 1.00 (s, 3H-29), 0.99 (brs, 3H-30); ^{13}C NMR ($CDCl_3$) and DEPT 135 ($CDCl_3$) spectra (see Figures S13 and S14): 77.2 (C-1), 43.0 (C-2), 215.4 (C-3), 39.6 (C-4), 48.0 (C-5), 21.1 (C-6), 25.9 (C-7), 47.9 (C-8), 19.8 (C-9), 26.1 (C-10), 26.5 (C-11), 32.9 (C-12), 45.3 (C-13), 48.8 (C-14), 35.5 (C-15), 28.6 (C-16), 52.2 (C-17), 18.0 (C-18), 29.8 (C-19), 41.1 (C-20), 18.1 (C-21), 34.8 (C-22), 25. (C-23), 145.8 (C-24), 126.4 (C-25), 171.7 (C-26), 12.0 (C-27), 19.3 (C-28), 25.4 (C-29), 21.2 (C-30).

3.3. Microbiological assay (see supplement)

4. Conclusions

Phytochemical studies of propolis *n*-hexane extract led to the isolation of a new cycloartane-type triterpenoid, 1-hydroxymangiferonic acid (**2**) along with seven other known compounds: mangiferonic acid (**1**); mangiferolic acid (**3**); 27-hydroxymangiferolic acid (**4**), isolated for the first time from propolis; 27-hydroxymangiferonic acid (**5**); *α*-amyrin (**6**); *β*-amyrin (**7**) and lupeol (**8**). The hexane crude extract and mixture of compounds **6**, **7** and **8** were tested for their antimicrobial activity against some Gram- positive and Gram-negative bacteria. Thus, the observed biological activity of propolis *n*-hexane extract may be due to the synergistic action of the isolated compounds. Based on its antibacterial properties, propolis *n*-hexane extract may be used as ointment for the management of skin infections or as a complementary therapy during respiratory infections. Further studies are required to evaluate the safety and clinical utility of propolis in humans.

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References

Abd Jalil MA, Kasmuri AR, Hadi H. 2017. Stingless bee honey, the natural wound healer: a review. Skin Pharmacol Physiol. 30(2):66–75. doi:10.1159/000458416.

Alanazi S. 2022. Antineoplastic and antitrypanosomal properties of propolis from *Tetragonula biroi* Friese. Molecules. 27(21):7463. doi:10.3390/molecules27217463.

Alenezi SS, Alenezi ND, Ebiloma GU, Natto M, Ungogo MA, Igoli JO, Ferro VA, Gray AI, Fearnley J, de Koning HP, et al. 2022. The antiprotozoal activity of papua new guinea propolis and its triterpenes. Molecules. 27(5):1622.

Almuhayawi MS. 2020. Propolis as a novel antibacterial agent. Saudi J Biol Sci. 27(11):3079–3086. doi:10.1016/j.sjbs.2020.09.016.

Alorfi HS. 2022. Antifungal–antiproliferative norcycloartane-type triterpenes from the Red Sea green alga *Tydemania expeditionis*. Open Chem. 20(1):912–919. doi:10.1515/chem-2022-0179.

Anjaneyulu V, Satyanarayana P, Viswanadham KN, Jyothi VG, Rao KN, Radhika P. 1999. Triterpenoids from *mangifera indica* part iii in the series triterpenoids from *mangifera indica*. Phytochemistry. 50(7):1229–1236.

Balderas-Cordero D, Canales-Alvarez O, Sánchez-Sánchez R, Cabrera-Wrooman A, Canales-Martinez MM, Rodriguez-Monroy MA. 2023. Anti-inflammatory and histological analysis of skin wound healing through topical application of mexican propolis. Int J Mol Sci. 24(14):11831. doi:10.3390/ijms241411831.

Bankova V, Popova M, Trusheva B. 2018. The phytochemistry of the honeybee. Phytochem. 155:1–11. doi:10.1016/j.phytochem.2018.07.007.

Béji-Srairi R, Younes I, Snoussi M, Yahyaoui K, Borchard G, Ksouri R, Frachet V, Wided MK. 2020. Ethanolic extract of Tunisian propolis: chemical composition, antioxidant, antimicrobial and antiproliferative properties. J Apic Res. 59(5):917–927. doi:10.1080/.00218839.2020.1732572.

Centers for Disease Control and Prevention. 2013. Antibiotic resistance threats in the United States. https://www.cdc.gov/ drugresistance/pdf/ar-threats-2013-508.

Choi H, Park J-S, Kim K-M, Kim M, Ko K-W, Hyun C-G, Ahn JW, Seo J-H, Kim S-Y. 2018. Enhancing the antimicrobial effect of genistein by biotransformation in microbial system. J Ind Eng Chem. 63:255–261. doi:10.1016/j.jiec.2018.02.023.

Choudhari MK, Punekar SA, Ranade RV, Paknikar KM. 2012. Antimicrobial activity of stingless bee (*Trigona sp.*) propolis used in the folk medicine of Western Maharashtra, India. J Ethnopharmacol. 141(1):363–367. doi:10.1016/j.jep.2012.02.047.

Chuttong B, Lim K, Praphawilai P, Danmek K, Maitip J, Vit P, Wu M-C, Ghosh S, Jung C, Burgett M, et al. 2023. Exploring the functional properties of propolis, geopropolis, and cerumen, with a special emphasis on their antimicrobial effects. Foods. 12(21):3909. doi:10.3390/foods12213909.

Devequi-Nunes D, MaCHado BAS, Barreto G, Rebouças Silva J, da Silva DF, da Rocha JLC, Brandão HN, Borges VM, Umsza-Guez MA, Lightfoot DA. 2018. Chemical characterization and biological activity of six different extracts of propolis through conventional methods and supercritical extraction. PLoS One. 13(12):e0207676. doi:10.1371/journal.pone.0207676.

Donlan RM. 2021. Biofilms and device-associated infections - Volume 7, Number 2—April 2001 - emerging infectious diseases journal - CDC. [cited 2021 Sep 15]. https://wwwnc.cdc.gov/eid/article/7/2/70-0277_article.

El-Guendouz S, Aazza S, Lyoussi B, Bankova V, Popova M, Neto L, Faleiro ML, Miguel MdG 2018. Moroccan Propolis: a natural antioxidant, antibacterial, and antibiofilm against Staphylococcus aureus with no induction of resistance after continuous exposure. Evid Based Complement Alternat Med. 2018(1):9759240. doi:10.1155/2018/9759240.

Escobedo-Martínez C, Concepción Lozada M, Hernández-Ortega S, Villarreal ML, Gnecco D, Enríquez RG, Reynolds W. 2012. ^{1}H and ^{13}C NMR characterization of new cycloartane triterpenes from *Mangifera indica*. Magnetic Reson in Chemistry. 50(1):52–57.

Fitzpatrick MC, Bauch CT, Townsend JP, Galvani AP. 2019. Modelling microbial infection to address global health challenges. Nat Microbiol. 4(10):1612–1619. doi:10.1038/s41564-019-0565-8.

Freitas MO, Ponte FAF, Lima MAS, Silveira ER. 2008. Flavonoids and triterpenes from the nest of the stingless bee trigona spinipes. J Braz Chem Soc. 19(3):532–535.

Gajdács M. 2019. The Continuing Threat of Methicillin-Resistant Staphylococcus aureus. Antibiotics (Basel, Switzerland) 8(2):52. doi:10.3390/antibiotics8020052

Hernández-Vázquez L, Palazon J, Navarro-Ocaña A. 2012. The Pentacyclic Triterpenes, α,β-amyrins: A Review of Sources and Biological Activities, Phytochemicals - A Global Perspective of Their Role in Nutrition and Health, Dr Venketeshwer Rao (Ed.), ISBN: 978-953-51-0296-0, InTech, Available from: http://www.intechopen.com/books/phytochemicals-a-global-perspective-of-their-role-innutrition-andhealth/the-pentacyclic-triterpenes-amyrins-a-review-of-sources-and-biological-activities

Jabir SM, Sulaiman MG, Taqi JZ, Li D. 2018. Chemical characterization of Iraqi propolis samples and assessing their antioxidant potentials. Microbes Infect. 20(2):89–100. doi:10.1016/j.micinf.2017.10.007.

Jaqueline C, Uilson S, Paola R, Marcio D, Jose B, Claudia C. 2015. Antimicrobial, antioxidant, anti-inflammatory, and cytotoxic activities of propolis from the stingless Bee *Tetragonis cafiebrigi* (Jataí). Evid Based Complement Altern Med. 2015:1–11.

Kharsany K, Viljoen A, Leonard C, van Vuuren S. 2019. The new buzz: investigating the antimicrobial interactions between bioactive compounds found in South African propolis. J Ethnopharmacol. 238:111867. doi:10.1016/j.jep.2019.111867.

Kubat M, Karabulut Z, Şengül S. 2021. Effect of propolis on wound healing in sacrococcygeal pilonidal disease: a randomized controlled clinical trial. Pak J Pharm Sci. 34(3(Supplementary):1063–1067.

Kumar N, Mueen Ahmed KK, Dang R, Shivananda TN, Das K. 2009. GC-MS analysis of propolis of Indian origin. J Young Pharmacists. 1(1):46. doi:10.4103/0975-1483.51876.

Kuropatnicki AK, Szliszka E, Krol W. 2013. Historical aspects of propolis research in modern times. Evid Based Complement Alternat Med. 2013:964149. doi:10.1155/2013/964149.

Li F, Awale S, Zhang H, Tezuka Y, Esumi H, Kadota S. 2009. Chemical constituents of propolis from myanmar and their preferential cytotoxicity against a human pancreatic cancer cell line. J Nat Prod. 72(7):1283–1287.

Lotfy M, Badra G, Burham W, Alenzi F. 2006. Combined use of honey, bee propolis and myrrh in healing a deep, infected wound in a patient with diabetes mellitus. Br J Biomed Sci. 63(4):171–173. doi:10.1080/09674845.2006.11732742.

Mahamat AA, Nyemb JN, Gade IS, Ngenge AT, Talla E, Céline H, Sophie L, Mbafor JT. 2020. A New fatty acid and some triterpenoids from propolis of Nkambe (North-West Region, Cameroon) and evaluation of the antiradical scavenging activity of their extracts. Open Chemistry. 18(1):239–243. doi:10.1515/chem-2020-0016.

Marcucci MC. 1995. Propolis: chemical composition, biological properties and therapeutic activity. Apidologie. 26(2):83–99. doi:10.1051/apido:19950202.

Mello CBSB, Petrus CCJ, Hubinger DM. 2010. Concentration of flavonoids and phenolic compounds in aqueous and ethanolic propolis extracts through nanofiltration. J Food Eng. 96(4):533–539. doi:10.1016/j.jfoodeng.2009.08.040.

Miguel MG, Nunes S, Dandlen SA, Cavaco AM, Antunes MD. 2010. Phenols and antioxidant activity of hydro-alcoholic extracts of propolis from Algarve, South of Portugal. Food Chem Toxicol. 48(12):3418–3423.

Nguyen HX, Nguyen MTT, Nguyen NT, Awale S. 2017. Chemical constituents of propolis from vietnamese trigona minor and their antiausterity activity against the panc-1 human pancreatic cancer cell line. J Nat Prod. 80(8):2345–2352.

Peycheva S, Apostolova E, Gardjeva P, Peychev Z, Kokova V, Angelov A, Slavov A, Murdjeva M. 2019. Effect of Bulgarian propolis on the oral microflora in adolescents with plaque-induced gingivitis. Revista Brasileira De Farmacognosia. 29(3):271–277. doi:10.1016/j.bjp.2018.11.001.

Popova M, Silici S, Kaftanoglu O, Bankova V. 2005. Antibacterial activity of Turkish propolis and its qualitative and quantitative chemical composition. Phytomedicine. 12(3):221–228. doi:10.1016/j.phymed.2003.09.007.

Popova M, Trusheva B, Antonova D, Cutajar S, Mifsud D, Farrugia C, Tsvetkova I, Najdenski H, Bankova V. 2011. The specific chemical profile of Mediterranean propolis from Malta. Food Chem. 126(3):1431–1435. doi:10.1016/j.foodchem.2010.11.130.

Przybyłek I, Karpiński TM. 2019. Antibacterial properties of propolis. Molecules. 24(11):2047. doi:10.3390/molecules24112047.

Pujirahayu N, Suzuki T, Katayama T. 2019. Cycloartane-type triterpenes and botanical origin of propolis of stingless indonesian bee *Tetragonula sapiens*. Plants. 8(3):57. doi:10.3390/plants8030057.

Ristivojević P, Dimkić I, Trifković J, Berić T, Vovk I, Milojković-Opsenica D, Stanković S. 2016. Antimicrobial activity of serbian propolis evaluated by means of MIC, HPTLC, bioautography and chemometrics. PLoS One. 11(6):e0157097. doi:10.1371/journal.pone.0157097.

Rosandy AR, Ishak SSO, Sabri NA, Ahmad WYW, Al Muqarrabun LMR. 2021. Antibacterial activity of lupeol from the bark of Dehaasia cuneate (Lauraceae). Crbb. 2(2):145–148. doi:10.5614/crbb.2021.2.2/BOFY6724.

Sakava P, Tagatsing FM, Zeuko'o ME, Talla E, Laurent S, Hénoumont C, Van Der E, Awantu FA, Atchadé ADT, Mbafor TJ. 2021. Two new cycloartenol and some pentacyclic triterpenoids from cameroonian propolis (Ngaoundal, AD Region) and evaluation of their antimicrobial activity. Nat Prod Chem and Res. 9(8):1–11.

Sakava P, Talla E, Chelea M, Tchinda TA, Zeuko'o ME, Laurent S, Vander EL, Tagatsing FM, Yaya AJ, Atchade DTA, et al. 2014. Pentacyclic triterpenes and crude extracts with antimicrobial activity from Cameroonian brown propolis samples. J App Pharm Sci. 4(07):001–009.

Salatino A. 2022. Perspectives for uses of propolis in therapy against infectious diseases. Molecules. 27(14):4594. doi:10.3390/.molecules27144594.

Shabani M, Mokhtarian M, Kalbasi-Ashtari A, Kazempoor R. 2021. Effects of extracted propolis (*Apis mellifera*) on physicochemical and microbial properties of rainbow-trout fish burger patties. J Food Process Preserv. 45(12):e16027. doi:10.1111/jfpp.16027.

Silva BB, Rosalen LP, Cury AJ, Ikegaki M, Souza CV, Esteves A, Alencar MS. 2008. Chemical composition and botanical origin of red propolis, a new type of brazilian propolis. Evid Based Complement Alternat Med. 5(3):313–316. doi:10.1093/ecam/nem059.

Silva-Carvalho R, Baltazar F, Almeida-Aguiar C. 2015. Propolis: a complex natural product with a plethora of biological activities that can beexplored for drug development. Evid Based Complement Altern Med. 2015:1–29. doi:10.1155/2015/206439.

Sulaiman GM, Ad'hiah AH, Al-Sammarrae KW, Bagnati R, Frapolli R, Bello E, Uboldi S, Romano M, Panini N, Scanziani E, et al. 2012. Assessing the anti-tumour properties of Iraqi propolis *in vitro* and *in vivo*. Food Chem Toxicol. 50(5):1632–1641. doi:10.1016/j.fct.2012.01.022.

Sulaiman GM, Al Sammarrae KW, Ad'hiah AH, Zucchetti M, Frapolli R, Bello E, Erba E, D'Incalci M, Bagnati R. 2011. Chemical characterization of Iraqi propolis samples and assessing their antioxidant potentials. Food Chem Toxicol. 49(9):2415–2421. doi:10.1016/j.fct.2011.06.060.

Sun C, Wu Z, Wang Z, Zhang H. 2015. Effect of ethanol/water solvents on phenolic profiles and antioxidant properties of Beijing propolis extracts. Evi Based Comp Altern Med. 8:1–9.

Talla E, Tamfu AN, Gade IS, Yanda L, Mbafor JT, Laurent S, Elst LV, Popova M, Bankova V. 2017. New mono-ether of glycerol and triterpenes with DPPH radical scavenging activity from Cameroonian propolis. Nat Prod Res. 31(12):1379–1389. doi:10.1080/14786419.2016.1253077.

Tamfu NA, Ceylan O, Cârâc G, Talla E, Dinica MR. 2022. Antibiofilm and anti-quorum sensing potential of cycloartane-type triterpene acids from cameroonian Grassland Propolis: phenolic profile and antioxidant activity of crude extract. Molecules. 27(15):4872. doi:10.3390/molecules27154872.

Tran TD, Ogbourne SM, Brooks PR, Sánchez-Cruz N, Medina-Franco JL, Quinn RJ. 2020. Lessons from exploring chemical space and chemical diversity of propolis components. Int J Mol Sci. 21(14):4988.

Trusheva B, Trunkova D, Bankova V. 2007. Different extraction methods of biologically active components from propolis: a preliminary study. Chem Cent J. 1(1):13. doi:10.1186/1752-153X-1-13.

Vázquez LH, Palazon J, Navarro-Ocaña A. 2012. The pentacyclic triterpenes α, β-amyrins: a review of sources and biological activities, phytochemicals - a global perspective of their role in nutrition and health. Dr Venketeshwer Rao (Ed.), ISBN: 978-953-51-0296-0. www.intechopen.com. doi:10.5772/27253.

WHO. 2017. Global priority list of Antibiotic-resistant Bacteria to guide research, Discovery, and Development of New Antibiotics. https://www.who.int/medicines/publications/WHO-PPL-Short_Summary_25Feb-ET_NM_WHO.pdf.

WHO. 2019. Critically important antimicrobials for human medicine 6th revision 2018. Ranking of medically important antimicrobials for risk management of antimicrobial resistance due to non-human use. Cataloguing-in-Publication (CIP) data. CIP data are available at http://apps.who.int/iris.

Woźniak M, Mrówczyńska L, Waśkiewicz A, Rogoziński T, Ratajczak I. 2019. Phenolic profile and antioxidant activity of propolis extracts from Poland. Nat Prod Com. 14:1–7.

Zayyanu UU, Mohamed M. 2015. Analysis of phytochemical compounds in water and ethanol extracts of malaysian propolis. Int J Pharm Biol Sci. 6:374–380.