Chemistry & Biodiversity

WILEY-VCH

CHEMISTRY & BIODIVERSITY

RESEARCH ARTICLE

Comparative Assessment of Conventional and Sustainable Extraction Methods for Essential Oils and Phenolic Compounds From *Ammodaucus Leucotrichus*

Abdelhamid Neggad[1] | Farid Benkaci-Ali[1] | Sarah Garifo[2] | Sophie Laurent[2]

[1]Faculty of Chemistry, Laboratory of Functional Organic Analysis, University of Sciences and Technology Houari Boumediene, BP 32 El Alia Bab Ezzouar, Algiers, Algeria | [2]Unit of General, Organic and Biomedical Chemistry, NMR and Molecular Imaging Laboratory, University of Mons, Mons, Belgium

Correspondence: Farid Benkaci-Ali (benkaciaf@yahoo.fr)

Received: 29 April 2025 | **Revised:** 28 July 2025 | **Accepted:** 30 July 2025

Keywords: *Ammodaucus leucotrichus* | essential oils | hydrodistillation | microwave | polyphenols | steam distillation

ABSTRACT

The aim of this study was to identify the most suitable extraction methods for isolating essential oils and polyphenols from *Ammodaucus leucotrichus*. Essential oils were extracted using hydrodistillation (HD), microwave-assisted HD (MW-HD) and microwave-assisted steam distillation (MW-SD), while polyphenols were obtained via maceration (MAC), Soxhlet (SOX), ultrasonic extraction (ULTR), and microwave-assisted extraction (MW). HD yielded the highest amount of essential oil (1.95%), and GC/MS analysis revealed that perillaldehyde (56.80%–62.67%) and limonene (22.04%–62.50%) are the most abundant compounds in the essential oils obtained through all techniques. Although the microwave-assisted methods (MW-HD and MW-SD) resulted slightly lower yields, but they offer substantial advantages in terms of reduced extraction time, lower energy consumption, and preservation of essential oil quality. For polyphenol extraction, MW and ULTR techniques produced the richest extracts, with a concentration of 624.11 mg EAG/g at 917.73 mg EAG/g. LC/MS analysis of the polyphenol extracts revealed several bioactive compounds identified for the first time, including taxifolin (2.24–12.90 mg/g), rutin (10.25 mg/g), isorhamnetin (12.82–43.33 mg/g), and 3-hydroxyflavone (0.85–5.21 mg/g). DPPH radical scavenging assays revealed the high potent radical-scavenging activity of extracts, highlighting the potential of *A. leucotrichus* as a promising natural source of bioantioxidant.

1 | Introduction

Over centuries, humans have harnessed natural resources for food, cosmetics, clothing, and medicine. Among these, medicinal plants stand out for their diverse therapeutic applications. Today, the use of medicinal plants remains a key approach to health-care worldwide, largely due to their rich reservoir of bioactive secondary metabolites [1].

Algeria hosts remarkable floristic diversity, with around 3000 plant species distributed across several botanical families [2, 3]. Among these, the Apiaceae family includes *Ammodaucus leucotrichus*, commonly known as 'camel cumin' a lesser-known but ethno botanically significant species native to the Saharan regions of Algeria and Morocco. Traditionally, local populations have used *A. leucotrichus* seeds to treat digestive disorders, infections and inflammation [4]. Recent studies have revealed that this species is particularly rich in essential oils (EOs) and polyphenolic compounds, which contribute to its antioxidant, antimicrobial and anti-inflammatory properties [5, 6].

Despite its traditional significance, *A. leucotrichus* remains underexplored in modern phytochemical and pharmacological research, particularly concerning how extraction methods influ-

ence its chemical profile and bioactivity. Extraction plays a crucial role in isolating bioactive compounds, and the choice of technique can significantly affect both yield and composition. Conventional methods, such as hydrodistillation (HD) and maceration (MAC), are widely used due to their simplicity. However, novel green extraction techniques, including microwave-assisted and ultrasound-assisted extraction have attracted interest for their higher efficiency, reduced solvent consumption, and better preservation of thermolabile compounds [7].

In this context, the present study aimed to evaluate the effect of different extraction methods (both conventional and green techniques) on the yield and chemical composition of EOs and polyphenols extracted from *A. leucotrichus* seeds collected in the Bechar region (southwest Algeria). EOs were obtained using conventional HD, microwave-assisted HD (MW-HD), and microwave-assisted steam distillation (MW-SD), followed by GC–MS analysis. Polyphenols were extracted using ethanol and methanol as solvents and four different techniques: MAC, Soxhlet extraction (SOX), ultrasound-assisted extraction (ULTR) and microwave-assisted extraction (MW). The qualitative and quantitative profiles of phenolic compounds were determined by liquid chromatography/mass spectrometry (LC/MS), and the antioxidant activity of the extracts was subsequently evaluated.

This work seeks to provide a clearer understanding of how extraction strategies influence the recovery of bioactive compounds from *A. leucotrichus*, with potential implications for its future use in food, pharmaceutical and cosmetic applications.

2 | Results and Discussion

2.1 | EO Extraction Yield

The EO obtained from *A. leucotrichus* seeds by various techniques is distinguished by its blue colour and distinctive odour. The greatest EO yield (1.95% w/w) was obtained by HD, close to the yield reported by Khaldi et al. (2.15%) obtained by HD for Bechar region [8]. This value is higher than those reported by Alaoui et al. (1.5%) in the Tata region of southern Morocco [9], while they are lower than the results obtained in the Dakhla region of Western Sahara (2.72%) [10]. MW-SD resulted in a slightly reduced yield (1.78%), while the yield obtained by MW-HD was 1.34%. These findings are consistent with those reported by G. Crescente, who observed that steam distillation of *Mentha spicata* L. yielded a higher EO content (0.28%) compared to MW-HD (0.24%) [11].

The observed variation in EO yield can be attributed to distribution of EO compounds present in *A. leucotrichus* seeds. The majority of these compounds are located in exogenous sites, whereas MW-HD is a technique that is more efficient at extracting endogenous sites [12]. This explains comparatively modest yield attained by MW-HD in comparison to other techniques.

Although, HD yielded 1.95% (w/w) of EO, the yields obtained by MW-SD and MW-HD were slightly lower, at 1.78% and 1.34%, respectively. However, microwave-assisted methods offered a substantial advantage in terms of extraction time, requiring only 45 min for MW-SD and MW-HD compared to approximately 3 h for HD.

To further assess extraction efficiency, the energy consumption per gram of EO was calculated using the following formula:

$$E = (P \times t) / (3600 \times 1000 \times m_{EO})$$

where E is the energy consumed (kWh/g), P is the power of the device (W), t is the operating time (s) and m_{EO} is the mass of EO obtained (g).

This notable reduction in extraction time translated into significantly lower energy consumption and operational costs. Specifically, microwave-assisted extractions consumed only 0.58 kWh/g for MW-HD and 0.45 kWh/g for MW-SD, compared to 2.65 kWh/g for conventional HD. This quantitative analysis highlights the superior energy efficiency of microwave-assisted techniques.

2.2 | Effect of Extraction Methods on the Chemical Profile of EOs

Data from the GC–MS analysis of EOs from our samples (Table 1) indicate that perillaldehyde is the most abundant compound in oils extracted by the different techniques with a percentage ranging from 56.8% to 62.67%. The EO obtained by HD tended to show the highest proportion of perillaldehyde, although this difference was not statistically significant ($p = 0.055$). These findings are in close alignment with those observed in the EO derived from *A. leucotrichus* harvested at Debdeb, Illizi (southeastern Algeria) (60.1%) [13] and at Bechar (59.12%) [14].

Limonene is the second major compound, with a percentage between 20.44% and 26.5%. These values are in agreement with those found by Louail et al. in the Bechar region (23.89%) [14], exceeding the previous results found by Dahmane et al. in the Debdeb region (6.9%) [13].

In addition, δ-2-carene and methyl perillate are significant compounds, with percentages ranging from 6.5% to 8.69% and 2.4% to 3.21%, respectively. As demonstrated by the existing literature, these compounds have been identified in the EO of *A. Leucotrichus* in multiple regions, as evidenced by references [13, 14].

The main compounds identified in EO such as perillaldehyde have a multitude of applications, as an ingredient in perfumes, cosmetics and aromas. The substance exhibits a robust spicy, oily and herbaceous taste [15]. Perillaldehyde can be converted to perillyl alcohol, which is also employed in the manufacture of perfumes and exhibits a range of properties, including antidepressant [16], neuroprotective [17] and insecticide effects [18].

Limonene is used as an additive in a variety of commercial products, including cleaning agents, foodstuffs, perfumes and cosmetics. Furthermore, it is employed as an insecticide [19]. Limonene has been demonstrated to facilitate wound healing and anabolic processes. In addition, it has been shown to have beneficial effects on stress, depression, inflammation, oxidative stress, muscle cramps and viral infections. In addition, it exhibits a range of anti-cancer properties, as evidenced by studies [20, 21].

TABLE 1 | Chemical composition of *Ammodaucus leucotrichus* essential oils obtained by different techniques.

				HD	MW-HD	MW-SD
Classes	**Compounds**	**RI***	**RI_{cal}**		**A%**	
H. MONO	Tri-cyclene	926	919	0.64 ± 0.04^{b}	0.74 ± 0.06^{b}	0.93 ± 0.06^{a}
	Camphene	954	950	—	—	0.07 ± 0.0^{a}
	β-Pinene	979	976	0.51 ± 0.01^{b}	0.65 ± 0.03^{a}	0.69 ± 0.05^{a}
	α-Phellandrene	1002	990	0.48 ± 0.02^{b}	0.50 ± 0.03^{b}	0.60 ± 0.01^{a}
	δ-2-Carene	1002	999	6.50 ± 0.36^{b}	7.28 ± 0.41^{b}	8.69 ± 0.29^{a}
	p-Cymene	1024	1016	0.16 ± 0.0^{ab}	0.13 ± 0.02^{b}	0.19 ± 0.01^{a}
	Limonene	1029	1026	20.44 ± 1.05^{b}	22.04 ± 0.88^{b}	26.50 ± 1.24^{a}
	H. MONO %			**28.73 ± 1.48^{b}**	**31.35 ± 1.43^{b}**	**37.69 ± 1.66^{a}**
MONO OX	*cis*-Limonene oxide	1136	1126	—	0.19 ± 0.0^{a}	0.13 ± 0.01^{a}
	trans-Limonene oxide	1142	1131	—	0.34 ± 0.04^{a}	—
	neo-Dihydro carveol	1194	1190	0.12 ± 0.01^{b}	0.44 ± 0.03^{a}	—
	γ-Terpineol	1199	1211	0.35 ± 0.02^{a}	—	0.27 ± 0.01^{b}
		1241	1231	0.90 ± 0.10^{a}	1.08 ± 0.07^{a}	0.68 ± 0.04^{b}
	Car-3-en-2-one	1248	1253	0.51 ± 0.02^{a}	0.53 ± 0.03^{a}	0.42 ± 0.02^{b}
	Perillaldehyde	1271	1282	62.67 ± 2.62^{a}	58.85 ± 2.09^{a}	56.80 ± 2.33^{a}
	α-Terpinen-7-al	1285	1283	0.21 ± 0.01^{a}	—	—
	γ-Terpinen-7-al	1291	1290	0.14 ± 0.0^{a}	0.17 ± 0.02^{a}	—
	Perillyl alcohol	1295	1303	0.88 ± 0.04^{a}	0.94 ± 0.08^{a}	0.11 ± 0.01^{b}
	MONO OX %			**65.78 ± 3.01^{a}**	**62.54 ± 2.36^{ab}**	**58.41 ± 2.42^{b}**
H. SESQ	α-Copaene	1376	1370	0.10 ± 0.0^{a}	0.10 ± 0.01^{a}	0.10 ± 0.01^{a}
	β-Cubebene	1388	1384	—	—	0.10 ± 0.0^{a}
	Germacrene D	1481	1473	0.20 ± 0.03^{b}	0.33 ± 0.04^{a}	0.13 ± 0.01^{b}
	Bicyclogermacrene	1500	1489	0.12 ± 0.0^{a}	—	0.12 ± 0.0^{a}
	δ-Amorphene	1512	1517	0.10 ± 0.01^{a}	—	—
	H. SESQ %			**0.52 ± 0.04^{a}**	**0.43 ± 0.06^{a}**	**0.45 ± 0.02^{a}**
SESQ OX	Germacrene D-4-ol	1575	1569	0.20 ± 0.03^{a}	—	—
	Caryophyllene oxide	1583	1572	—	0.12 ± 0.01^{a}	—
	14-Hydroxy-(*Z*)-caryophyllene	1667	1656	0.12 ± 0.0^{a}	—	—
	Shyobunol	1689	1684	0.73 ± 0.06^{a}	0.49 ± 0.05^{b}	0.41 ± 0.02^{b}

(Continues)

TABLE 1 | (Continued)

Classes	Compounds	RI*	RI_{cal}	HD	MW-HD	MW-SD
					A%	
	SESQ OX %			**0.61 ± 0.06[b]**	**0.41 ± 0.02[c]**	
			1.06 ± 0.09[a]			
Others	Linalool formate	1216	1212	—	0.48 ± 0.04[a]	—
	2-Undecanone	1294	1293	—	0.31 ± 0.02[a]	0.11 ± 0.0[b]
	Methyl perillate	1393	1391	3.21 ± 0.13[a]	3.21 ± 0.09[a]	2.40 ± 0.16[b]
	Others %			**3.21 ± 0.13[b]**	**4.00 ± 0.15[a]**	**2.51 ± 0.16[c]**
	Total %			**99.30**	**98.92**	**99.46**

Note: Values are average ± standard deviation of three repetitions. Different lowercase letters in the same row indicate significant differences ($p < 0.05$); results ranked: a > b > c.
Abbreviations: H.MONO, monoterpenic hydrocarbons; H.SESQ, sesquiterpenic hydrocarbons; MONO.OX, oxygenated monoterpenes; RI*, Adams retention index; RI_{cal}, calculated retention index; SESQ.OX, oxygenated sesquiterpenes.

With regard to the chemical classes (Figure 1), oxygenated monoterpenes (MONO.OX) constitute the most abundant class in EO, exhibiting a percentage varying from 58.41% to 65.78%. The monoterpene hydrocarbons (H.MONO) constitute the second most prevalent group, with percentages ranging from 28.73% to 37.69%. The remaining two classes, oxygenated sesquiterpenes (SESQ.OX) and sesquiterpene hydrocarbons (H.SESQ), exhibited comparatively low percentages, ranging from 0.41% to 1.06% and from 0.43% to 0.52%, respectively.

As shown in Table 1, the high percentage in perillaldehyde was obtained by HD (62.67%), followed by MW-HD and MW-SD techniques, with 58.85% and 56.80% respectively. Although HD appeared to yield a relatively higher proportion of perillaldehyde, this difference was not statistically significant compared to other techniques ($p > 0.05$). By contrast, the limonene content showed significant differences between MW-SD and the other extraction methods ($p < 0.05$). The EO obtained by MW-SD exhibited the highest concentration of limonene (26.5%), followed by MW-HD (22.04%) and HD (20.44%).

The chemical profile of several compounds varies according to extraction techniques used and amongst all the compounds identified in Table 1, four are exclusively detected in EO extracted by HD, including α-terpinén-7-al (0.21%), germacrene D-4-ol (0.2%), 14-hydroxy-(*Z*)-caryophyllene and δ-amorphene (0.1%). A total of three compounds were specifically detected in the MW-HD volatile: linalool formate (0.48%), *trans*-limonene oxide (0.34%) and caryophyllene oxide (0.12%), whereas β-cubebene (0.1%) and camphene (0.07%) were exclusively isolated in MW-SD volatile.

As shown in Figure 1, the EO obtained by HD exhibits a higher proportion of oxygenated monoterpenes (MONO.OX: 65.78%), oxygenated sesquiterpenes (SESQ.OX: 1.06%), and sesquiterpene hydrocarbons (H. SESQ: 0.52%) compared to the other extracted oils, MW-SD (58.41%, 0.41% and 0.45% respectively) and MW-HD (62.54%, 0.61% and 0.43%.respectively), respectively. While the percentage of H.MONO was higher in the MW-SD EO (37.62%) than MW-SD (31.35%) and HD (28.73%) EOs.

The results presented above indicate that it is not feasible to universally prioritize one extraction technique over another in terms of quality, as each method selectively extracts specific compounds based on its operating principles. For instance, HD and MW-HD are more effective at extracting compounds located in endogenous sites, due to their ability to penetrate plant tissues through prolonged or internal heating. In contrast, MW-SD, where water vapours interact primarily with the plant surface, is more adept at extracting compounds located in exogenous sites. In addition, HD, which involves slow and uniform heating, favours the extraction of thermally stable and more polar compounds such as perillaldehyde. On the other hand, the rapid and localized heating of MW-SD and MW-HD facilitates the extraction of highly volatile compounds like limonene, which could otherwise degrade under extended thermal exposure [22]. These differences clearly demonstrate how the physical and thermal characteristics of each technique influence the selectivity, composition and preservation of EO constituents.

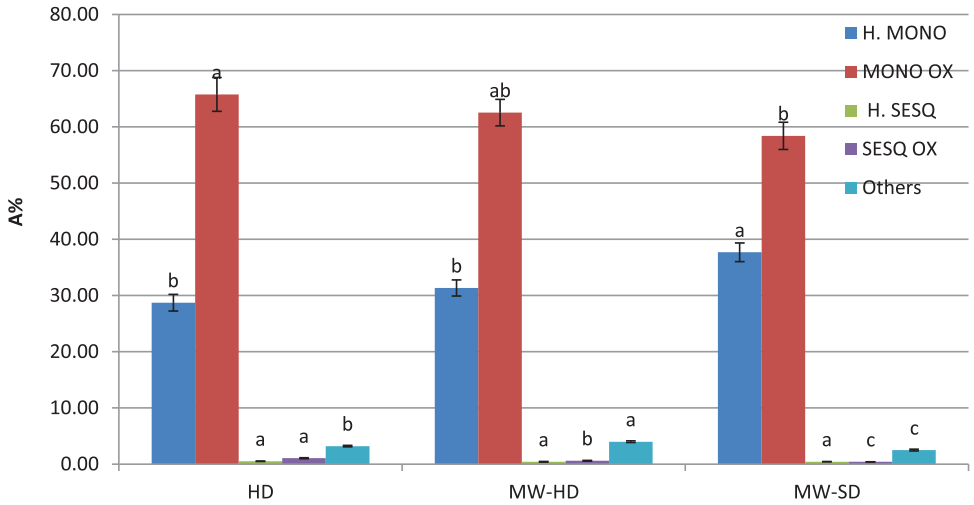

FIGURE 1 | Effect of different extraction techniques on chemical classes (H. MONO: monoterpene hydrocarbons, MONO.OX: oxygenated monoterpenes, H. SESQ: sesquiterpene hydrocarbons, SESQ OX: oxygenated sesquiterpenes, Others: other compounds, HD: hydrodistillation, MW-HD: microwave-assisted extraction, MW-SD: microwave-assisted steam distillation). Values are average ± standard deviation of three repetitions. Different lowercase letters in the same row indicate significant differences ($p < 0.05$); results ranked: a > b > c.

Furthermore, the use of microwaves in the extraction of EOs from *A. leucotrichus*, such as MW-HD and MW-SD, provides benefits over conventional HD with regard to time and energy savings. The application of powerful microwave radiation speeds up the extraction process, while causing no significant alteration to the composition of the EO [22].

The EOs extracted from *A. leucotrichus* seeds via various extraction methods are characterized by a predominance of specific compounds, namely perillaldehyde and limonene. However, the percentage of these compounds varies depending on the extraction technique employed. The EO obtained by HD exhibited the highest concentration of perillaldehyde (62.67%), while the EO obtained by MW-SD demonstrated the highest concentration of limonene (26.5%).

2.3 | Polyphenolic Extracts

This study examined the impact of extraction methods and solvents on the yield and phenolic composition of *A. leucotrichus*. Four techniques were used to extract phenolic compounds: MAC, SOX, ULTR, and microwave (MW). For each technique, ethanol and methanol were used as extraction solvents. Subsequently, the yield was calculated, the total polyphenol content was measured, and an LC–MS analysis was conducted.

The dry polyphenolic extracts were obtained after evaporation of the extraction solvent, and the yields determined as illustrated in Figure 2.

The yield of extracts obtained with ethanol ranged from 11.35% to 17.7%. The largest amounts were obtained by MAC (17.7%) and SOX (17.65%), while ULTR extraction and microwave-assisted gave yields of 14.83% and 11.35%, respectively. These findings are more pronounced than those previously documented by Mohammedi et al. on ethanolic extract (5.23%–14.45%) [23]. Also, the value found by MAC is only slightly greater than that reported by Belbachir, who obtained a yield of 11.92% using the same solvent and extraction technique [5].

For methanolic extracts, SOX extraction provided the most substantial yield (21.65%), followed by MW (13.9%), and then MAC (13.25%) and ULTR extraction (12.85%). These results are not in accordance with those reported by Sifi et al. on a methanolic extract using MAC as technique (20%) [24]. Moreover, the yield obtained by the SOX extraction is comparable to that reported by Manssouri (15.23%) using the same solvent and extraction technique [25]. Furthermore, according to Tambun et al. [26], microwave-assisted extraction provided the highest yield compared to conventional methods for *Annona muricata* extracts, highlighting the potential of this green technique for enhancing extraction efficiency. These results can be attributed to the elevated temperature of the SOX and MW extractions, which increases the solubility of the compounds in the solvent.

However, when employing the same extraction technique, the yields for SOX and MW with methanol produced greater quantities of extract (21.65% and 13.9%, respectively) than in ethanol (17.65% and 11.35%, respectively). Conversely, MAC and ULTR led to slightly larger amounts with ethanol (17.7% and 14.83%, respectively) compared to methanolic extracts (13.25% and 12.85%, respectively).

Although conventional methods such as MAC and SOX extraction provided slightly higher polyphenol yields, they required significantly longer extraction times, 48 h for MAC and 3 h for SOX. In contrast, green extraction techniques, including ULTR extraction and microwave-assisted extraction, produced acceptable amounts of extract in only about 45 min. This notable reduction in extraction time highlights the practical advantages of these modern techniques, especially in industrial applications, even if the quantities obtained remain somewhat more modest than those achieved with conventional methods [27].

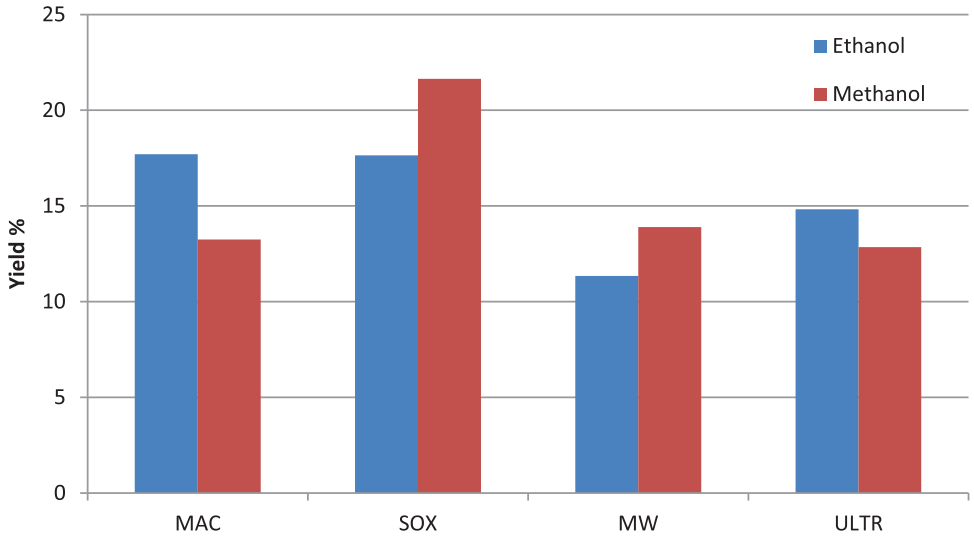

FIGURE 2 | Variation in the extraction yield of *Ammodaucus leucotrichus* polyphenolic extracts using different methods and solvents (MAC: maceration, SOX: Soxhlet, ULTR: ultrasonic extraction, MW: microwave-assisted extraction).

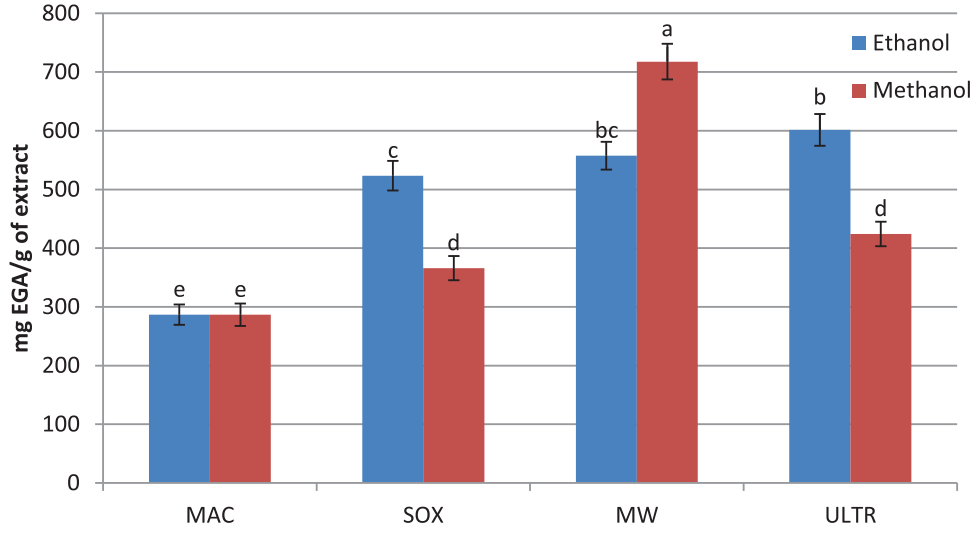

FIGURE 3 | Variation in total polyphenol content of different *Ammodaucus leucotrichus* extracts (MAC: maceration, SOX: Soxhlet, ULTR: ultrasonic extraction, MW: microwave-assisted extraction). Values are average ± standard deviation of three repetitions. Different lowercase letters in the same row indicate significant differences ($p < 0.05$); results ranked: a > b > c > d > e.

2.4 | Total Polyphenol Content

The results of the total polyphenol content are illustrated in Figure 3. The findings indicate that the total polyphenol content of *A. leucotrichus* extracts is contingent upon the extraction technique and the solvent utilized. The extracts exhibited a high richness in polyphenolic compounds Among the various extraction methods, those obtained by MW demonstrated the highest polyphenol content, for both solvents methanol (717.73 mg EGA/g) and ethanol (557.44 mg EGA/g), followed by ULTR (601.41 and 424.11 mg EGA/g), while SOX extraction ranked third (523.40 and 365.95 mg EGA/g). MAC extraction, on the other hand, exhibited the lowest polyphenol content (286.71 and 286.52 mg EGA/g). These results are higher than those reported by Mohammedi [23]. Moreover, these consistent with those reported by Veršić Bratinčević, who found that microwave-assisted extraction of sea fennel yielded extracts with higher polyphenol concentrations compared to other extraction techniques [28].

Based on these observations, it can be concluded that the microwave and ultrasound techniques are more efficient for the extraction of polyphenols from *A. leucotrichus* than other techniques. This can be attributed to the integration of thermal, mechanical and physical–chemical processes in these two techniques, which enhance the destruction of plant structures and facilitate the release of phenolic compounds. Where, in MW, rapid internal heating generates localized pressure within plant cells, leading to cell wall rupture and improved solubilization of intracellular compounds. Similarly, ULTR extraction relies on acoustic cavitation, which induces shear forces and micro jets that disrupt cellular structures, thereby

facilitating the diffusion of phenolic compounds into the solvent [28].

In contrast, methanol extraction yielded a higher concentration of polyphenols (286.71–717.73 mg EGA/g) than ethanol (286.52–557.44 mg EGA/g) across all four extraction methods.

It can be hypothesized that methanol is a more suitable solvent for extracting phenolic compounds from *A. leucotrichus*. This may be explained by the solubility of polyphenols in each solvent. Overall, the results for total polyphenol content were higher than those reported by Mohammedi [23].

2.5 | LC/MS Analysis of Phenolic Extracts

To study the influence of extraction methods and solvents on the phenolic composition of *A. leucotrichus*, four techniques (MAC, SOX, ULTR and microwave) and two solvents (ethanol and methanol) were tested. Then the isolated extracts were analysed by LC–MS.

For compound identification, a comparison was made between the retention times and mass spectra of chromatographic peaks and those of the standards utilized. As demonstrated in Table 2, of the 34 standard compounds analysed, 10 were identified including phenolic acids as caffeic acid (23.67 min), *o*-anisic acid (28.42 min), *m*-anisic acid (31.31 min), ferulic acid (28.5 min) and 3,4,5-trimethoxy *trans*-cinnamic acid (31.97 min). Flavonoid analysis revealed the presence of the following compounds: taxifolin (29.49 min), rutin (32.61 min), quercetin (41.73 min), isorhamnetin (45.87 min) and 3-hydroxyflavone (47.4 min) were detected.

It is noteworthy that hydroxyl ferulic acid and quercetin detected in all samples examined except in MAC, these compounds have previously been identified by other authors in the ultrasonic extract of *A. leucotrichus* [29]. Ferulic acid has been shown a variety of beneficial effects, including anti-carcinogenic, cardioprotective, antioxidant, neuroprotective, hepatoprotective and anti-inflammatory properties [30, 31]. It has been demonstrated that quercetin exerts a salutary effect on human health, particularly as an antioxidant, in the treatment of allergies, asthma, atopic diseases and cancer. This review also describes the interactions of these compounds with other drugs and their associated side effects [32].

The other compounds were identified for the first time in phenolic extracts of *A. leucotrichus*. Most of these compounds have demonstrated potential pharmacological advantages, in particular, taxifolin has shown noteworthy inhibitory properties against various pathological processes, such as inflammation, malignant tumours, microbial infections, oxidative stress, cardiovascular disease and liver disease [33]. In addition, rutin has been shown to possess a range of pharmacological activities, including antioxidant, cytoprotective, vasoprotective, anticarcinogenic, neuroprotective and cardioprotective effects [34]. Isorhamnetin has also been demonstrated to have a number of beneficial effects, including preventing cardiovascular and cerebrovascular disease, anti-tumour activity, anti-inflammatory properties, antioxidant effects, the protection of organs and the prevention of obesity [35]. Finally, 3-hydroxyflavone has been demonstrated to exhibit a variety of pharmacological activities, including antiviral, anti-tumour, anti-inflammatory, anticholinesterase, cytotoxicity and particularly high antioxidant activity [36].

As illustrated in Table 2, two compounds were exclusively detected in the extracts obtained by SOX. The first, *o*-anisic acid, was detected only in the methanolic extract at a low concentration of 0.11 mg/g of extract, while the other, rutin, appeared only in the methanolic extract obtained by SOX with a quantity of 10.25 mg/g of extract.

On the other hand, caffeic acid, ferulic acid and 3,4,5-trimethoxy *trans*-cinnamic acid were identified in higher concentrations in the ethanolic extracts obtained by different methods, with the exception of microwave technique, where these compounds were found in higher concentrations in the methanolic extract (9.94; 17.95 and 6.83 mg/g, respectively). In contrast, taxifolin exhibited high concentrations in the methanolic extracts, except in microwave technique (7.74 mg/g). Furthermore, isorhamnetin was identified with significant concentrations in ethanolic extracts, except when using SOX, a higher quantity in the methanolic extract (32.43 mg/g) was noted.

2.5.1 | Impact of Extraction Techniques on Phenolic Composition

The extraction of polyphenols with ethanol enabled the identification of nine compounds in total, with seven compounds being identified in the extracts obtained through MAC and microwave, and eight compounds being identified in extracts obtained by SOX and ultrasound methods.

The phenolic acids identified show higher quantities in ethanolic extracts obtained by MAC and ultrasound than those obtained by microwave and SOX; such as caffeic acid which presents a quantity of 13.33 mg/g of extract in MAC and 7.22 mg/g in ULTR, whereas it presents only 4.18 mg/g in SOX and 2.53 mg/g in MW. The same observation for ferulic acid (MAC-Eth: 27.38 mg/g, ULTR-Eth: 26.23 mg/g, SOX-Eth: 11.14 mg/g and MW-Eth: 3.42 mg/mL), *m*-anisic acid (MAC-Eth: 31.65 mg/g, ULTR-Eth: 12.85 mg/g, SOX-Eth: 9.71 mg/g and MW-Eth: 6.69 mg/mL) and 3,4,5-trimethoxy *trans*-cinnamic acid (MAC-Eth: 9.38 mg/g, ULTR-Eth: 12.45 mg/g, SOX-Eth: 5.31 mg/g and MW-Eth: 3.42 mg/mL). This may be explained by the fact that MAC and ULTR are distinguished by their thermal gentleness (especially MAC) and their efficiency in releasing thermosensitive polar compounds. However, microwave (MW) and SOX involve more intense or prolonged heating, which may result in the degradation or partial isomerization of these sensitive phenolic acids [28].

For flavonoids, when ethanol is used, taxifolin, quercetin and isorhamnetin were detected in all four techniques used, the highest amount of taxifolin being extracted by microwave (7.74 mg/g), while the lowest was recorded in MAC (2.24 mg/g), which may be explained by the ability of microwaves to rapidly disrupt cell walls, releasing less polar or encapsulated compounds. Quercetin and isorhamnetin showed higher percentages when ultrasound was used, at 29.93 and 43.33 mg/g respectively, probably due to

TABLE 2 | Phenolic compounds composition in methanolic and ethanolic extract of *Ammodaucus leucotrichus* according to the different techniques used.

Compounds	Concentration (mg/g of extract)								*m/z* $[M+Na]^+$, $[M+H]^+$ fragments
	Ethanol				Methanol				
	MAC	MW	SOX	ULTR	MAC	MW	SOX	ULTR	
Caffeic acid	13.33 ± 2.13[a]	2.53 ± 0.21[c]	4.18 ± 0.37[c]	7.21 ± 0.51[b]	8.15 ± 0.53[b]	9.94 ± 0.87[b]	—	4.01 ± 0.31[c]	181–163
o-Anisic acid	—	—	—	—	—	—	0.11 ± 0.02[a]	—	175–153–135
Ferulic acid	27.38 ± 3.04[a]	3.42 ± 0.19[d]	11.14 ± 0.16[c]	26.23 ± 3.11[a]	—	17.95 ± 1.15[b]	3.78 ± 0.21[d]	10.12 ± 0.97[c]	195–177
Taxifolin	2.24 ± 0.26[d]	7.74 ± 0.61[b]	4.91 ± 0.36[c]	3.68 ± 0.31[cd]	11.32 ± 0.93[a]	5.83 ± 0.25[c]	12.90 ± 1.01[a]	11.81 ± 0.95[a]	327–305
m-Anisic acid	31.65 ± 2.82[a]	6.69 ± 0.48[d]	9.71 ± 0.45[c]	12.85 ± 0.84[c]	19.20 ± 1.36[b]	29.34 ± 3.07[a]	0.73 ± 0.04[e]	19.33 ± 1.70[b]	153
3,4,5-Trimethoxy *trans*-cinnamicacid	9.38 ± 0.56[b]	6.62 ± 0.39[c]	5.31 ± 0.26[c]	12.45 ± 1.09[a]	6.80 ± 0.36[c]	6.83 ± 0.40[c]	—	9.11 ± 0.55[b]	239–221
Rutin	—	—	10.25 ± 0.82[a]	—	—	—	—	—	611–303
Quercetin	25.50 ± 3.03[a]	17.19 ± 1.57[b]	25.92 ± 2.85[a]	29.93 ± 3.17[a]	—	8.07 ± 0.52[c]	12.30 ± 1.16[c]	7.26 ± 0.48[c]	303
Isorhamnetin	22.30 ± 1.92[c]	12.82 ± 1.22[d]	24.74 ± 2.14[c]	43.33 ± 3.76[a]	—	—	32.43 ± 3.81[b]	22.56 ± 2.74[c]	317
3-Hydroxy flavonone	5.41 ± 0.33[b]	—	—	7.21 ± 0.46[a]	—	—	0.85 ± 0.05[c]	—	261–239

Note: Values are average ± standard deviation of three repetitions. Different lowercase letters in the same row indicate significant differences ($p < 0.05$); results ranked: a > b > c > d > e.
Abbreviations: MAC, maceration; MW, microwave-assisted extraction; SOX, Soxhlet; ULTR, ultrasonic extraction.

the cavitation effect, which improves diffusion without thermal degradation [28]. However, 3-hydroxyflavonone was only identified in MAC (5.41 mg/g) and in ULTR (7.21 mg/g), this further underlines the need for mild conditions for certain compounds. Finaly, rutin was only detected in the ethanolic extract obtained by SOX (10.25 mg/g), indicating good thermal stability of this compound [37].

As shown in Table 2, nine compounds were identified in methanolic extracts, four in MAC, six in MW, seven in SOX and ULTR. The analysis revealed the presence of two distinct components, *o*-anisic acid and 3-hydroxyflavonone exclusively in SOX with concentrations of 0.11 and 0.85 mg/g, respectively. In contrast, was detected in substantial amounts in both SOX (32.43 mg/g) and ULTR (22.56 mg/g) but not found with MAC and MW techniques.

Caffeic acid and 3,4,5-trimethoxy *trans*-cinnamic acid was not detected in SOX-methanolic extract. Similarly, ferulic acid and quercetin were not detected in MAC-methanolic extract. However, when methanol was used as solvent, the optimal extraction of caffeic acid and ferulic acid was observed in MW (9.94 mg/g and 17.95, respectively), 3,4,5-trimethoxy *trans*-cinnamic acid in ULTR (9.11 mg/g) and quercetin in SOX (12.3 mg/g). For taxifolin, the highest concentration was observed in SOX (12.9 mg/g), while the richest extract of *m*-anisic acid was isolated by MW (29.34 mg/g).

These results highlight the critical role of the extraction method in determining selectivity of phenolic compounds. MAC and ultrasound, due to their gentle conditions, are more suitable for preserving thermolabile and polar phenolic acids. In contrast, microwave and SOX techniques, while less effective for certain acids, appear to be more efficient in extracting specific flavonoids such as taxifolin and rutin, especially when combined with methanol. The interaction between extraction parameters (time, temperature, energy input) and the chemical nature of the compounds (polarity, thermal stability) explains the observed variability.

Therefore, the selection of extraction technique depends on the solvent used. This could be explained by the boiling points of the two solvents, where that of ethanol (78.7°C) is higher than that of methanol (64.7°C). Consequently, heat-sensitive compounds are less exposed to heat when using methanol.

2.5.2 | Impact of Extraction Solvents on Phenolic Composition

For extracts obtained by MAC, four compounds were detected only in the ethanolic extract: ferulic acid (27.3 mg/g), quercetin (25.5 mg/g), isorhamnetin (22.3 mg/g) and 3-hydroxyflavonone (5.41 mg/g). On the other hand, four additional compounds were identified in the ethanol and methanol extracts, including caffeic acid, *m*-anisic acid and 3,4,5-trimethoxy *trans*-cinnamic acid, extracted more efficiently using ethanol. Conversely, taxifolin presents a higher quantity in the methanolic extract.

The application of SOX extraction allowed the identification of eight compounds in the ethanolic extract and seven in the methanolic extract. Caffeic acid (4.18 mg/g), 3,4,5-trimethoxy *trans*-cinnamic acid (5.31 mg/g) and rutin (10.25 mg/g) were detected only in the ethanolic extract, while o-anisic acid (0.11 mg/g) and 3-hydroxyflavonone (0.85 mg/g) appeared exclusively in the methanolic extract. Other compounds were identified in both extracts, such as ferulic acid, *m*-anisic acid and quercetin, which exhibited enhanced extraction by ethanol. As for the taxifolin and isorhamnetin demonstrated higher concentrations when methanol was utilized.

When ULTR was used, a total of eight compounds were identified in the ethanolic extract and seven in the methanolic extract, including 3-hydroxyflavonone (0.85 mg/g) appearing only in ethanol. The other seven compounds were identified in both solvents, with caffeic acid, ferulic acid, 3,4,5-trimethoxy *trans*-cinnamic acid, quercetin and isorhamnetin showing higher concentrations in the ethanolic extract, while taxifolin and *m*-anisic acid were best extracted in methanol.

For the microwave technique MW (Table 2), seven compounds were identified using ethanol and six by methanol. The composition of the two extracts differed in that isorhamnetin (12.83 mg/g) was identified only in the ethanolic extract. In regard to the remaining compounds, taxifolin and isorhametin were found in higher concentrations in the ethanolic extract, while caffeic acid, ferulic acid, *m*-anisic acid and 3,4,5-trimethoxy *trans*-cinnamic acid were found in higher quantities in methanolic extracts.

2.6 | DPPH Radical Scavenging Assay

The anti-free radical activity was quantified as the percentage reduction in initial DPPH uptake by polyphenolic extracts at various concentrations, with the objective of determining the IC_{50} for each extract. Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) were used as reference antioxidants. The IC_{50} results are displayed in Figure 4.

Overall, all the extraction techniques tested exhibited high potent DPPH free radical scavenging activity for both solvents (Figure 4), with IC_{50} values ranging from 1.12 to 1.87 mg/mL. When comparing the techniques with each other, statistical analysis showed that they mostly belonged to the same group, except for MW extracts, which formed a distinct group, suggesting that MW yielded the most active extracts. In general, these results are comparable to BHT (1.75 mg/mL), whereas BHA demonstrated a much stronger antioxidant effect (0.24 mg/mL). On the other hand, these results are close to those reported by Mohammedi for the ethanolic extract [23].

It should be noted that the DPPH assay, while widely used for preliminary screening, only measures the ability to scavenge a specific stable free radical and does not fully reflect the overall antioxidant potential of the extracts. This constitutes a limitation of the present study.

Consequently, it can be concluded that *A. leucotrichus* extracts possess a high anti-radical capacity and it can be suggested as a bioantioxidant of choice for their use in various agri-food and cosmetic fields.

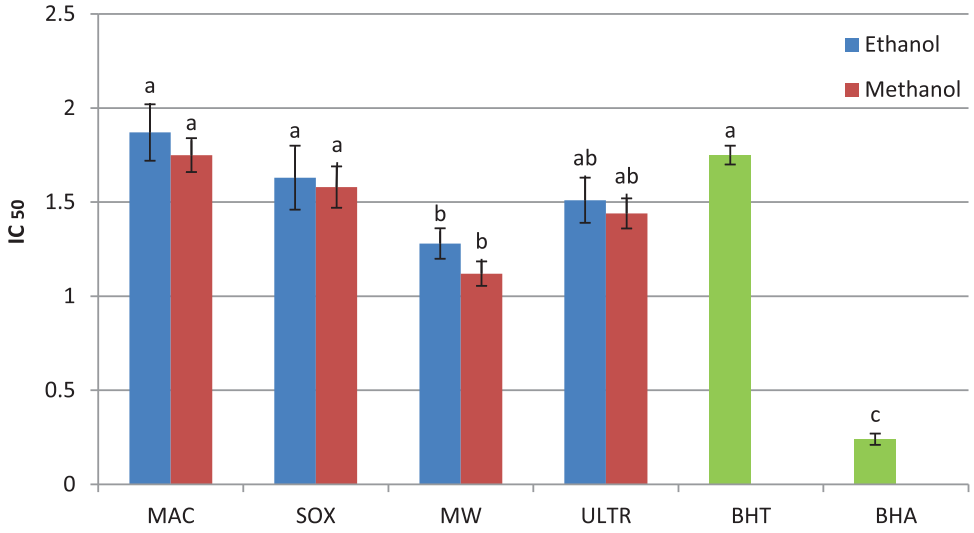

FIGURE 4 | DPPH radical scavenging assay of *A. Leucotrichus* extracts by 2,2-diphenyl-1-picrylhydrazyl expressed by IC_{50} (MAC: maceration, SOX: Soxhlet, ULTR: ultrasonic extraction, MW: microwave-assisted extraction). Values are average ± standard deviation of three repetitions. Different lowercase letters in the same row indicate significant differences ($p < 0.05$); results ranked: a > b > c.

3 | Conclusions

The results show that among the tested techniques for extracting EOs from *A. leucotrichus*, conventional HD achieved a yield with 1.95%, followed by MW-SD (1.78%) and MW-HD (1.34%). Despite slightly lower yields, microwave-assisted methods notably reduced extraction time and energy consumption, which is advantageous for industrial applications. In terms of composition, all methods produced oils with similar major constituents, though HD favoured perillaldehyde (62.67%) while MW-SD enhanced limonene extraction (26.5%), highlighting the selectivity of each method.

For polyphenol extraction, microwave-assisted extraction (MW) and ultrasound-assisted extraction (ULTR) led to the highest total polyphenol contents (557.44 and 601.41 mg GAE/g, respectively). These outcomes can be attributed to rapid internal heating in MW and acoustic cavitation in ULTR, both improving cell disruption and diffusion. LC/MS analysis identified for the first time in this species bioactive compounds such as taxifolin (2.24–12.90 mg/g), rutin (10.25 mg/g), isorhamnetin (12.82–43.33 mg/g) and 3-hydroxyflavone (0.85–5.21 mg/g).

Furthermore, the data reveal that milder techniques like MAC and ultrasound better preserve thermo-sensitive and polar phenolic acids, especially in ethanolic extracts, while microwave and SOX methods are more efficient in extracting certain flavonoids like taxifolin and rutin, particularly when methanol is used. These differences reflect the combined effects of extraction parameters (temperature, duration, solvent type) and the physicochemical properties of the target compounds.

It can be concluded that the optimal extraction technique depends on the nature of the compounds targeted: each method shows a preference for certain families of compounds. Nevertheless, green techniques such as microwave and ultrasound-assisted extraction emerge as promising alternatives, combining efficiency with reduced time and energy consumption.

Finally, anti-free radical assays show that *A. leucotrichus* polyphenolic extracts possess strong radical scavenging potential, comparable to standard antioxidants BHT and BHA, due to their richness in bioactive molecules identified by LC/MS.

These findings underline the potential of combining traditional knowledge of local plants with sustainable extraction technologies to develop natural bioactive compounds for use in food, cosmetic, and pharmaceutical industries.

4 | Experimental Section

4.1 | Chemicals

The chemicals and reagents employed in this study are sodium carbonate (Na_2CO_3) and 1,1-diphenyl-2-picrylhydrazine (DDPH), BHA and BHT were procured from Sigma-Aldrich (USA). Gallic acid was procured from Merck (Germany), while Folin–Ciocalteu reagent was obtained from VWR (USA).

The alkane mixture, comprising alkanes with carbon numbers between 6 and 28, was procured from Sigma-Aldrich (USA).

The analytical-grade organic solvents, namely methanol and ethanol, were procured from VWR (USA). The HPLC-grade solvents, comprising ultrapure water was obtained via a Milli-Q system (Millipore, Bedford, USA), methanol from VWR (USA), and acetic acid from Sigma-Aldrich (USA).

For the LC–MS standards, a total of 32 phenolic compound standards were obtained. The standards included 3,5-dimethoxycinnamic, cinnamic, ferulic, *trans*-2,4-dimethoxycinnamic, gallic, *m*-anisic, *o*-anisic, syringic, iso-vanillic, isoferulic, 3,4,5-trimethoxybenzoic and 3,4,5-trimethoxycinnamic acids; 3-hydroxyflavone; caffeine; quercetin and imperatorin from Sigma-Aldrich (USA), tannic, sinapic, caffeic and ascorbic acids, catechin, orcinol, rutin, coumarin

and taxifolin from Fluka (Germany), and chlorogenic and vanillic acids, baicalein, daidzein, isorhamnetin, myricetin and kaempferol from Extrasynthese (France).

4.2 | Plant Material

A total of 2 kg of seeds of *A. leucotrichus* were collected in April 2023 following the seed formation period in Bechar (Taghit region, southwestern Algeria, 30°55′0″ N and 2°1′60″ W). Fifty individuals samples were collected from a 5-km radius. The samples were then dried in the shade for a period of 15 days, then the seeds were ground to a diameter of between 0.5 and 2 mm using a seed grinder, and utilized for extraction. Plant identification was confirmed by Pr Mohamed Hazzit from National Higher School of Agronomy (ENSA, Algiers). Reference specimens have been deposited in the ENSA herbarium under accession number AL12042023.

4.3 | EO Extraction Methods

The present study investigated the influence of extraction technique on the yield and composition of EOs from *A. leucotrichus*. To this end, three different extraction methods were utilized.

The extraction yield (R%) of EOs was calculated as the ratio of the mass of extracted oil (M_{EO}) to the mass of dry plant material (M_P), expressed as a percentage:

$$R\% = [M_{EO}/M_P] \times 100$$

4.3.1 | Conventional HD

The Clevenger-type device is used for HD. First, 50 g of plant material and 500 mL of water are introduced into a balloon. After installing the balloon in the balloon heater, switch it on and set the heating to optimum. The total extraction time is estimated at 3 h [38].

4.3.2 | MW-HD

Fifty grams of ground plant material was placed in a balloon with 500 mL of water. Then, the balloon was placed in a microwave oven and combined with the Clevenger apparatus. The microwave power was set to 450 W and the extraction time was 45 min [39].

4.3.3 | MW-SD

MW-SD was carried out using a microwave laboratory oven Milestone ETHOS X oven (Milestone, Italy). A flask filled with 500 mL is placed inside the microwave oven, while a vertical glass column packed with 50 g of plant material was positioned outside the oven and connected to a condenser. Water vapor begins to rise and pass through the plant material. Then the water vapor loaded with EO is condensed using a condenser and collected in a decanted ampoule. The extraction was performed for 45 min at a constant microwave power of 450 W [40].

The extracted EOs were dried over anhydrous sodium sulphate and then stored in glass vials protected from light in a refrigerator at 4°C before GC–MS analysis.

4.4 | Extraction Methods of Phenolic Compounds

For the extraction of polyphenols, four different techniques were employed. Each extraction technique was performed twice: once using methanol and once using ethanol as the solvent. The extraction yield (R%) of the polyphenolic extract was calculated using the following formula:

$$R\% = \text{(Weight of dry extract obtained (g) /Weight of dry plant material used (g))} \times 100$$

4.4.1 | Extraction by MAC

Twenty grams of plant material were placed in an Erlenmeyer flask containing 200 mL of solvent and agitated mechanically at room temperature for 48 h [41]. During the extraction process, the suspension was placed in a shaded area and hermetically sealed to prevent solvent evaporation and phenolic compound reaction with light.

4.4.2 | SOX Extraction

The SOX extraction method was employed, wherein 20 g of seeds of *A. leucotrichus* were placed in a cellulose cartridge and situated within a SOX extractor. The cartridge was connected to a balloon containing 200 mL of the extraction solvent. The heating process was conducted using a balloon heater with an extraction time of 3 h [42].

4.4.3 | ULTR

A 20 g mass of plant material was extracted with 200 mL of extraction solvent in a balloon. The flask was placed in an ultrasonic bath (DAIHAN Scientific, model WUC-D10H), operating at a frequency of 40 kHz without heating. The extraction was carried out for 45 min at room temperature [43].

4.4.4 | Microwave-Assisted Extraction (MW)

In this technique, a microwave oven was utilized to heat a balloon containing 20 g of *A. leucotrichus* and 200 mL of solvent. The balloon was connected to a cooler to prevent solvent loss. The microwave oven was calibrated at a power output of 450 W, and the extraction time was 45 min [44].

At the end of each extraction, the mixture was filtered on filter paper to obtain a solution enriched with phenolic compounds. The extracts were then subjected to vacuum evaporation, a rapid solvent removal process, using a rotary evaporator. The resulting residues were weighed to calculate the extraction yield and stored at 4°C in shaded, hermetically sealed flasks for a few days (maximum 1 week) before being subjected to further analyses. From the extracts obtained, the total polyphenols were determined, LC–MS analyses were performed, and the DPPH free radical scavenging activity was evaluated.

4.5 | Analysis of EOs

In this phase, gas chromatography with flame ionization detection (GC/FID) was employed for the quantification of compounds, while gas chromatography with mass spectrometry (GC/MS) was utilized for the identification of EO compounds.

4.5.1 | Gas Chromatography (GC/FID) Analysis

Gas chromatography (GC) analysis was conducted on a standard model 6890 series instrument (Agilent Technologies, Palo Alto, CA, USA) standard model, using the following conditions: fused-silica capillary column with a non-polar stationary phase HP5–MS (60 m, 0.25 mm i.d., 0.25 μm film, 5% biphenyl, 95% dimethylpolysiloxane). The detector utilized was a flame ionization detector (FID), the injector temperature set at 208°C and the detector temperature at 300°C. The initial oven temperature was set at 60°C for a duration of 10 min, thereafter, the oven temperature was increased from 60°C to 250°C at a rate of 4°C/min, the temperature was then maintained at 250°C for a period of 10 min. Helium was utilized as carrier gas, with a flow rate of 1 mL/min (0.03 MPa).

4.5.2 | GC/MS Analysis

Analysis of EOs was performed using a 6890 series gas chromatograph (Agilent Technologies) coupled to an MSD 5973 mass spectrometry (GC/MS) detector. The HP5-MS fused silica capillary column (60 m × 0.25 mm × 0.25 mm × 0.25 μm film thickness) was utilized for chromatographic peak separation. Helium was used as the carrier gas at an initial rate of 1 mL/min. The initial oven temperature was set at 60°C for 8 min; thereafter the oven temperature was increased at 250°C with a rate of 4°C/min, then 10 min of isothermal system. The injector was configured to operate in splitless mode and at a temperature of 280°C. The MS was operated in positive EI mode at 70 eV and a mass range of 50–550 m/z.

Compounds were identified by comparison of their GC retention indices (RI) on the HP5-MS column. This index was determined with reference to a homologous series of C6–C28 *n*-alkanes and to those of authentic standards available in the authors' laboratory. The identification process was further validated through a comparison of the compounds mass spectral fragmentation patterns with those stored in the database (Wiley/NBS library) and in the literature [45]. For each analysis, the peak area of each compound was determined by integrating the total ion current.

4.6 | Analysis of Polyphenolic Extracts

In this section, the total polyphenol content was determined using the Folin–Ciocalteu method. Subsequently, an LC/MS analysis was employed for the identification and quantification of phenolic compounds present in the extracts. For this purpose, polyphenol standard solutions were prepared by dissolving 1 mg of each reference in 1 mL of methanol. Initially, each reference was analysed individually by LC–MS. Subsequently, a series of standard mixtures were prepared and injected into the LC–MS. For the quantitative study, different concentrations of the standards were prepared to generate calibration curves for each standard.

4.6.1 | Total Polyphenol Content

The total polyphenol content was determined using the Folin–Ciocalteu method. This reagent is a yellow acid composed of a mixture of phosphotungstic and phosphomolybdic acids [46].

A volume of 20 μL of extract solution was mixed with 100 μL of Folin–Ciocalteu reagent and 1580 μL of water. After a period of 8 min, 300 μL of a 7.5% sodium carbonate solution was then added. Following, 120-min incubation in the dark, the absorbance was measured at 765 nm by UV–visible spectrophotometry, employing a blank solution comprising the solvent utilized for extract solubilization. Subsequently, the polyphenol concentration was subsequently calculated from the gallic acid calibration curve and expressed in mg of gallic acid equivalent per a gram of extract (mg GAE/g extract) [47]. The total phenolic content (TPC) was determined using the equation above.

$$TPC\,(mgGAE/g\,extract) = (C \times V)/M$$

where C is the concentration of gallic acid from the calibration curve (mg/mL), V the volume of extract used in the reaction (mL) and M the mass of plant material extracted (g).

4.6.2 | LC/MS Analysis

The analysis of phenolic compounds was conducted using a Waters Alliance LC-MS instrument. The instrumentation comprised a Waters Alliance 2695 HPLC and mass detector (ACQUITY QDa detector, Waters), with control provided by Masslynx v4.2 software. The separation process was carried out using a C18 XBridge column with a diameter of 4.6 mm, a length of 150 mm, and a particle size of 3.5 μm. The mobile phase was constituted by a solution of 1% acetic acid in ultrapure water (Mobile Phase A) and methanol (Mobile Phase B). The elution gradient program was as follows: at the start of the analysis, the mobile phase was composed of 15% B. This proportion was increased to 20% B at 10 min, 40% B at 20 min, 60% B at 30 min, 80% B at 45 min and 80% B at 50 min. At the start of the experiment, the mobile phase consisted of 80% B. The flow rate was 0.5 mL/min, the injection volume was 10 μL, the temperature of the auto-sampler was 25°C, and the column temperature was 30°C [48].

The mass spectrometer (QDa, Waters) was operated in positive scan mode. The capillary voltage was set at 0.8 kV, the cone voltage was 10 V, the source temperature was 600°C, and the desolvation gas flow was 800 L/h. The mass scan was conducted in the range of m/z 100–1000 with a sampling rate of 5 scans/s in scan mode. The analyses were performed using the external standard calibration method.

4.7 | DPPH Radical Scavenging Assay

The antioxidant potential of polyphenolic extracts was determined using the most widely used free radical scavenging assay: the 2,2-diphenyl-picryl-hydrazyl (DPPH) assay. All experiments were performed in triplicate for the different concentrations of each honey extract.

DPPH radical scavenging assay was carried out for each extract, where 25 µL of varying concentrations were combined with 975 µL of DPPH solution at a concentration of 60 µmol/L (0.0024 g in 100 mL of methanol). Subsequently, the mixture was incubated in the dark for 30 min, after which the absorbance was measured at 517 nm. The absorbance of the control solution (DPPH, 60 µmol/L) had already been determined at the same wavelength [49, 50]. The inhibition percentage of the DPPH free radical (I %) was calculated by using the following formula:

$$\%Inhibition = [(A_0 - A_t)/A_0] \times 100$$

where A_0 is the absorbance of the control (DPPH solution without sample) and A_t the absorbance of the test sample after reaction with DPPH

The IC_{50} was determined for each extract, defined as the concentration exhibiting 50% activity. The outcomes were then compared with the IC_{50} of the antioxidants BHT and BHA, which were utilized as standards.

4.8 | Statistical Analysis

All analyses and antioxidant activity measurements were performed in triplicate. Data are presented as means ± standard deviation (SD). Differences between groups were evaluated using ANOVA, followed by Tukey's post hoc test, performed with Jamovi software (version 2.6.26). A $p < 0.05$ was considered statistically significant.

Author Contributions

Abdelhamid Neggad: performed the experiments, analysed the data and wrote the draft of the article. **Farid Benkaci-Ali**: A. provided and identified the plant material, wrote and revised the manuscript. **Sarah Garifo**: performed the chromatographic analyses, established the data and revised the manuscript. **Sophie Laurent**: contributed to the materials in the experiments, performed the chromatographic analyses and revised the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. G. Rizzo, M. Disante, A. Mencarelli, et al., "The Farnesoid X Receptor Promotes Adipocyte Differentiation and Regulates Adipose Cell Function In Vivo," *Molecular Pharmacology* 70 (2006): 1164–1173.

2. F. Haddouchi, H. A. Lazouni, A. Meziane, and A. Benmansour, "Etude Physicochimique Et Microbiologique De L'huile Essentielle De *Thymus Fontanesii Boiss* & Reut," *Afrique Science: Revue Internationale des Sciences et Technologie* 5, no. 2 (2009): 246–259.

3. R. Soulimani, C. Younos, J. Fleurentin, F. Mortier, R. Misslin, and F. Derrieux, "Recherche de L'activité Biologique De *Melissa officinalis* L. sur le Système Nerveux Central De la Souris In Vivo et Le Duodenum De Rat In Vitro," *Plantes Médicinales Et Phytothérapie* 26 (1993): 77–85.

4. E. Idm'hand, F. Msanda, and K. Cherifi, "Medicinal Uses, Phytochemistry and Pharmacology of *Ammodaucus leucotrichus*," *Clinical Phytoscience* 6 (2020): 6.

5. F. Z. Belbachir, L. Hamdan Ramdani, C. Decombat, et al., "Chemical Profile and Assessment of Antioxidant, Anti-Inflammatory, and Antiproliferative Effects of *Ammodaucus leucotrichus* Coss. & Dur. Fruit Extracts on Human Breast Cancer," *Natural Products Communications* 19, no. 3 (2024): 1934578X241239827.

6. A. Hajib, I. Nounah, A. Oubihi, et al., "Chemical Composition and Biological Activities of Essential Oils From the Fruits of *Cuminum cyminum* L. and *Ammodaucus leucotrichus* L. (Apiaceae)," *Journal of Essential Oil-Bearing Plants* 23 (2020): 474–483.

7. A. N. Shikov, I. Y. Mikhailovskaya, I. A. Narkevich, E. V. Flisyuk, and O. N. Pozharitskaya, "Methods of Extraction of Medicinal Plants," in *Evidence-Based Validation of Herbal Medicine*, ed. P. K. Mukherjee (Elsevier, 2022), 771–796.

8. A. Khaldi, B. Meddah, A. Moussaoui, and P. Sonnet, "Anti-Mycotoxin Effect and Antifungal Properties of Essential Oil From *Ammodaucus leucotrichus* Coss. & Dur. on *Aspergillus flavus* and *Aspergillus ochraceus*," *Journal of Essential Oil-Bearing Plants* 20 (2017): 36–44.

9. A. Moulay, S. Brahim, B. Satrani, et al., "Bioactivity and Chemical Quality of *Ammodaucus leucotrichus* ssp. *leucotrichus* Coss. & Durieu Essential Oils From Morocco," *Natural Products* 10, no. 6 (2014): 208–214.

10. A. Velasco-Negueruela, M. J. Pérez-Alonso, P. L. P. De Paz, J. Palá-Paúl, and J. Sanz, "Analysis by Gas Chromatography–Mass Spectrometry of the Volatiles From the Fruits of *Ammodaucus leucotrichus* subsp. *leucotrichus* and subsp. Nanocarpus Grown in North Africa and the Canary Islands, Respectively," *Journal of Chromatography A* 1108 (2006): 273–275.

11. G. Crescente, G. Cascone, A. Sorrentino, M. G. Volpe, F. Boscaino, and S. Moccia, "Influence of Extraction Techniques on Chemical Composition, Antioxidant and Antifungal Activities of *Mentha spicata* L. Essential Oil: A Comparative Study of Microwave-Assisted Hydrodistillation and Steam Distillation," *Food Bioscience* 69 (2025): 106939.

12. H.-W. Wang, Y.-Q. Liu, S.-L. Wei, Z.-J. Yan, and K. Lu, "Comparison of Microwave-Assisted and Conventional Hydrodistillation in the Extraction of Essential Oils From Mango (*Mangifera indica L.*) Flowers," *Molecules* 15 (2010): 7715–7723.

13. D. Dahmane, T. Dob, S. Krimat, A. Nouasri, H. Metidji, and A. Ksouri, "Chemical Composition, Antioxidant and Antibacterial Activities of the Essential Oils of Medicinal Plant *Ammodaucus leucotrichus* From Algeria," *Journal of Essential Oil Research* 29 (2017): 48–55.

14. Z. Louail, A. Kameli, T. Benabdelkader, K. Bouti, K. Hamza, and S. Krimat, "Antimicrobial and Antioxidant Activity of Essential Oil of *Ammodaucus leucotrichus* Coss. and Dur. Seeds," *Journal of Materials and Environmental Science* 7 (2016): 2328–2334.

15. F. Erhunmwunsee, C. Pan, K. Yang, Y. Li, M. Liu, and J. Tian, "Recent Development in Biological Activities and Safety Concerns of

Perillaldehyde From Perilla Plants: A Review," *Critical Reviews in Food Science and Nutrition* 62 (2022): 6328–6340.

16. Y. Song, R. Sun, Z. Ji, X. Li, Q. Fu, and S. Ma, "Perilla Aldehyde Attenuates CUMS-Induced Depressive-Like Behaviors via Regulating TXNIP/TRX/NLRP3 Pathway in Rats," *Life Sciences* 206 (2018): 117–124.

17. Y. Qiu, X. Xue, G. Liu, et al., "Perillaldehyde Improves Cognitive Function In Vivo and In Vitro by Inhibiting Neuronal Damage via Blocking TRPM2/NMDAR Pathway," *Chinese Medicine* 16 (2021): 136.

18. C. You, Y. Wang, W. Zhang, et al., "Chemical Constituents and Biological Activities of the *Purple perilla* Essential Oil Against *Lasioderma serricorne*," *Industrial Crops and Products* 61 (2014): 331–337.

19. J. J. Chebet, J. E. Ehiri, D. J. McClelland, D. Taren, and I. A. Hakim, "Effect of D-Limonene and Its Derivatives on Breast Cancer in Human Trials: A Scoping Review and Narrative Synthesis," *BMC Cancer* 21 (2021): 902.

20. J. de Vasconcelos, C. Braz, F. O. de Carvalho, et al., "Mechanism of Action of Limonene in Tumor Cells: A Systematic Review and Meta-Analysis," *Current Pharmaceutical Design* 27 (2021): 2956–2965.

21. H. G. de Araújo-Filho, J. F. Dos Santos, M. T. B. Carvalho, et al., "Anticancer Activity of Limonene: A Systematic Review of Target Signaling Pathways," *Phytotherapy Research* 35 (2021): 4957–4970.

22. S. Périno-Issartier, C. Ginies, G. Cravotto, and F. Chemat, "A Comparison of Essential Oils Obtained From Lavandin via Different Extraction Processes: Ultrasound, Microwave, Turbohydrodistillation, Steam and Hydrodistillation," *Journal of Chromatography A* 1305 (2013): 41–47.

23. H. Mohammedi, "Extraction et Caractérisation Physico-Chimique Des Métabolites Secondaires de Ruta L., *Ammodaucus leucotrichus* et *Bassia muricata* L." (doctoral diss.), (2019).

24. I. Sifi, F. Z. Benaddou, and M. Yousfi, "Antioxidant and Antimicrobial Activities of Phenolic Extracts of Endemic Plants Marrubium Deserti and *Ammodaucus leucotrichus* From Algeria," *World Academy of Science, Engineering and Technology Bioengineering and Life Sciences International Science Index* 2 (2015): 12661.

25. M. Manssouri, M. Znini, and L. Majidi, "Studies on the Antioxidant Activity of Essential Oil and Various Extracts of *Ammodaucus leucotrichus* Coss. & Dur. Fruits From Morocco," *Journal of Taibah University for Science* 14 (2020): 124–130.

26. R. Tambun, V. Alexander, and Y. Ginting, "Performance Comparison of Maceration Method, Soxhletation Method, and Microwave-Assisted Extraction in Extracting Active Compounds From Soursop Leaves (*Annona muricata*): A Review," *IOP Conference Series Materials Science and Engineering* 1122, no. 1 (2021): 12095.

27. S. .F. Garofalo, F. Demichelis, V. Peletti, L. Picco, T. Tommasi, and D. Fino, "Comparative Study of Polyphenol Extraction Using Physical Techniques and Water as a Solvent: A Sustainable Approach for the Valorization of Apple Pomace," *Environmental Science and Pollution Research* (2024).

28. M. Veršić Bratinčević, R. Kovačić, M. Popović, S. Radman, and I. .G. Mekini, "Comparison of Conventional and Green Extraction Techniques for the Isolation of Phenolic Antioxidants From *Sea fennel*," *Processes* 11 (2023): 2172.

29. I. Es-Safi, H. Mechchate, A. Amaghnouje, et al., "Defatted Hydroethanolic Extract of *Ammodaucus leucotrichus* Cosson and *Durieu Seeds*: Antidiabetic and Anti-Inflammatory Activities," *Applied Science* 10 (2020): 9147.

30. E.-R. Jo, C. K. Youn, Y. Jun, and S. I.l Cho, "The Protective Role of Ferulic Acid Against Cisplatin-Induced Ototoxicity," *International Journal of Pediatric Otorhinolaryngology* 120 (2019): 30–35.

31. S. Ou and K. Kwok, "Ferulic Acid: Pharmaceutical Functions, Preparation and Applications in Foods," *Journal of the Science of Food and Agriculture* 84 (2004): 1261–1269.

32. G. S. Kelly, "Quercetin," *Alternative Medicine Review* 16 (2011): 172–194.

33. A. Das, R. Baidya, T. Chakraborty, A. K. Samanta, and S. Roy, "Pharmacological Basis and New Insights of Taxifolin: A Comprehensive Review," *Biomedicine & Pharmacotherapy* 142 (2021): 112004.

34. A. Ganeshpurkar and A. K. Saluja, "The Pharmacological Potential of Rutin," *Saudi Pharmaceutical Journal* 25 (2017): 149–164.

35. G. Gong, Y.-Y. Guan, Z.-L. Zhang, et al., "Isorhamnetin: A Review of Pharmacological Effects," *Biomedicine & Pharmacotherapy* 128 (2020): 110301.

36. B. Butun, G. Topcu, and T. Ozturk, "Recent Advances on 3-Hydroxyflavone Derivatives: Structures and Properties," *Mini-Reviews in Medicinal Chemistry* 18 (2018): 98–103.

37. J. L. d. S. Carvalho, M. M. Cunico, J. d. F. G. Dias, M. D. Miguel, and O. G. Miguel, "Termoestabilidade de Processos Extrativos de *Nasturtium officinale* R. Br., Brassicaceae por Sistema Soxhlet Modificado," *Quimica Nova* 32 (2009): 1031–1035.

38. J. Bruneton, *Pharmacognosie, Phytochimie, Plantes Médicinales*, 3th ed. (Lavoisier, 1999).

39. M. E. Lucchesi, F. Chemat, and J. Smadja, "Solvent-Free Microwave Extraction of Essential Oil From Aromatic Herbs: Comparison With Conventional Hydro-Distillation," *Journal of Chromatography A* 1043 (2004): 323–327.

40. C. Bouchachia, F. Benkaci-Ali, G. Eppe, and G. Scholl, "Effect of Different Parameters on Composition of Volatile Components of *Myristica fragrans* Seeds Extracted by Hydrodistillation Assisted by Microwave and Head-Space Solid-Phase Micro-Extraction," *Journal of Essential Oil Research* 29 (2017): 481–493.

41. N. A. Kamarudin, M. Markom, and J. Latip, "Effects of Solvents and Extraction Methods on Herbal Plants *Phyllanthus niruri*, *Orthosiphon stamineus* and *Labisia pumila*," *Indian Journal of Science and Technology* 9 (2016): 1–5.

42. O. R. Alara, N. H. Abdurahman, and C. I. Ukaegbu, "Soxhlet Extraction of Phenolic Compounds From *Vernonia cinerea* Leaves and Its Antioxidant Activity," *Journal of Applied Research on Medicinal and Aromatic Plants* 11 (2018): 12–17.

43. K. Rajbhar, H. Dawda, and U. Mukundan, "Polyphenols: Methods of Extraction," *Scientific Reviews and Chemical Communications* 5 (2015): 1–6.

44. X. Pan, G. Niu, and H. Liu, "Microwave-Assisted Extraction of Tea Polyphenols and Tea Caffeine From Green Tea Leaves," *Chemical Engineering and Processing: Process Intensification* 42 (2003): 129–133.

45. R. P. Adams, *Identification of Essential Oil Components bygas Chromatography/Mass Spectroscopy*, 4th ed. (Allured Publication, 2007).

46. V. L. Singleton, "Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent," *Methods in Enzymology* 299 (1999): 152–178.

47. Z. Ghouila, S. Laurent, C. Henoumont, L. Vander Elst, R. N. Muller, and A. Baaliouamer, "Rich Extract on Total Polyphenols and Antioxidant Activity Obtained by Conventional and Non-Conventional Methods From Ahmeur Bouamer Grape Seed," *Journal of Fundamental and Applied Sciences* 8 (2016): 692–711.

48. A. Neggad, F. Benkaci-Ali, S. Laurent, and G. Ayata, "A New Method of Extracting Polyphenols From Honey Using a Biosorbent Compared to the Commercial Resin Amberlite XAD2," *Journal of Separation Science* 44 (2021): 2089–2096.

49. L. A. Figueroa, L. B. Navarro, M. P. Vera, and V. L. Petricevich, "Antioxidant Activity, Total Phenolic and Flavonoid Contents, and Cytotoxicity Evaluation of *Bougainvillea xbuttiana*," *International Journal of Pharmacy and Pharmaceutical Sciences* 6 (2014): 497–502.

50. W. Brand-Williams, M.-E. Cuvelier, and C. Berset, "Use of a Free Radical Method to Evaluate Antioxidant Activity," *LWT-Food Science and Technology* 28 (1995): 25–30.