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Green engineering approaches in the comparative evaluation of essential oil extraction techniques from *Origanum* and *Mentha*

Miranda Misini[1], Fatmir Faiku[1*], Ilirjana Osmani[1,2], Arben Haziri[1], Aurel Nuro[2]

[1] Department of Chemistry, University of Prishtina, Rr. Agim Ramadani 3, 10000 Prishtina, Kosova
[2] Department of Chemistry, University of Tirana, Boulevard Zogu I, 1000 Tirana, Albania
* Corresponding author's e-mail: fatmir.faiku@uni-pr.edu

ABSTRACT

In the context of sustainable development and green engineering, this study investigated and compared environmentally friendly extraction techniques for obtaining the essential oils from *Origanum* and *Mentha* species. The essential oils were extracted using hydrodistillation (HD), supercritical CO_2 extraction (SC-CO_2), ultrasound-assisted extraction (UAE), and headspace solid phase microextraction (HS-SPME), employing "captured" solvents of different polarity (hexane and toluene). Chemical characterization was performed using GC-FID for quantitative analysis and GC-MS for compound identification. The results demonstrated that the extraction technique significantly affects both qualitative and quantitative profiles, with 30–120 compounds identified depending on the method. In *Mentha spp*., oxygenated monoterpenes dominated, particularly menthone (up to 42.6%) and menthol (up to 38.5%), with SC-CO_2 providing the highest selectivity and recovery. In contrast, the UAE extracts were enriched in monocyclic monoterpenes such as α-phellandrene (up to 42.18%) and limonene (up to 25.29%). For *Origanum* spp., thymol was the main compound, reaching 83.9% in the SC-CO_2 extracts, compared to 63.57% in HD and 53.15% in UAE. HS-SPME predominantly captured highly volatile hydrocarbons, including α-pinene (up to 29.06%), reflecting its suitability for aroma profiling, rather than total extraction. Solvent polarity, which was used as "captured solvent, further influenced extraction efficiency, with hexane favoring non-polar hydrocarbons and toluene slightly enhancing oxygenated compounds. In conclusion, the SC-CO_2 extraction proved to be the most effective and selective method, ensuring high yields and preservation of thermolabile bioactive compounds in both plant species. UAE represents a rapid and energy efficient alternative for hydrocarbon rich fractions, while HS-SPME is optimal for volatile profiling. These findings highlight the critical role of method selection in optimizing extraction strategies tailored to specific phytochemical targets and industrial applications.

Keywords: *Mentha*, *Origanum*, essential oils, extraction methods, supercritical CO_2 extraction, hydrodistillation, ultrasound-assisted extraction, HS-SPME, chemical profiling, volatile compounds.

INTRODUCTION

Aromatic and medicinal plants, such as oregano (*Origanum* spp.) and mint (*Mentha* spp.), are widely recognized for their rich content of essential oils and bioactive compounds, which exhibit significant antimicrobial, antioxidant, and pharmacological properties. These compounds are primarily composed of terpenes, terpenoids, and phenolic constituents such as thymol, carvacrol, and menthol, whose qualitative and quantitative profiles strongly depend on the extraction technique applied (Yousaf *et al*., 2021; Shoukair *et al*., 2024). Consequently, the selection of an appropriate extraction method is crucial for obtaining high-quality extracts and for accurately characterizing the chemical profile of these plants.

Hydrodistillation remains one of the most traditional and widely used methods for essential oil extraction due to its simplicity and effectiveness in isolating volatile compounds from plant matrices. However, this technique often requires long extraction times and may lead to thermal degradation or loss of thermolabile constituents (Chemat *et al*., 2020; Ameer *et al*., 2023). In contrast, modern extraction techniques, such as supercritical

CO_2 extraction, have gained increasing attention as green and efficient alternatives. Supercritical CO_2 offers certain, advantages including low toxicity, high selectivity, and the ability to preserve thermosensitive compounds due to operation at moderate temperatures (Herzyk *et al*., 2024). Furthermore, extraction conditions, such as pressure and temperature, significantly influence the yield and composition of the extracted essential oils (Xu *et al*., 2011).

Ultrasound-assisted extraction (UAE) is another innovative technique that enhances mass transfer through cavitation phenomena, leading to cell wall disruption and improved release of intracellular compounds. This method has been shown to reduce extraction time while maintaining, or even improving yield and quality compared to conventional techniques (Shoukair *et al*., 2024; Özdemir *et al*., 2025). Additionally, UAE is considered an environmentally friendly approach due to its lower energy consumption and solvent requirements (Manjarrez-Quintero *et al*., 2024).

Headspace (HS) techniques, particularly when coupled with gas chromatography mass spectrometry (GC-MS), provide a powerful tool for the analysis of volatile compounds without the need for extensive sample preparation. The HS methods enable the characterization of aroma profiles by selectively analyzing volatile fractions, often revealing differences in composition compared to traditional extraction methods (Burzynski-Chang *et al*., 2018). These differences highlight the importance of method selection when evaluating the chemical profile of plant extracts.

In recent years, increasing environmental concerns and the need for sustainable resource management have intensified the focus on green engineering approaches in the extraction of bioactive compounds from plant materials. Conventional techniques such as hydrodistillation and steam distillation, although widely used, are often associated with high energy consumption and potential thermal degradation of sensitive compounds. In contrast, advanced methods, such as supercritical CO_2 extraction and ultrasound-assisted extraction, represent environmentally friendly alternatives, offering reduced solvent use, lower energy requirements, and improved preservation of thermolabile constituents. These green technologies not only enhance extraction efficiency but also minimize environmental impact, making them highly relevant within the framework of ecological engineering. Therefore, the comparative evaluation of these techniques is essential for identifying sustainable and efficient strategies for essential oil extraction.

Recent studies emphasize that no single extraction method is universally superior; instead, each technique selectively extracts different classes of compounds depending on their polarity, volatility, and stability. Therefore, comparative studies are essential to determine the most effective extraction approach for specific plant species and target compounds (Chemat *et al*., 2012). In this context, oregano and mint serve as ideal models due to their well-documented phytochemical diversity and industrial relevance.

The aim of this study was to comparatively investigate the chemical profiles of the *Origanum* spp. and *Mentha* spp. extracts obtained using four different extraction techniques: hydrodistillation, supercritical CO_2 extraction, ultrasound assisted extraction, and headspace analysis. By evaluating the efficiency of each method in extracting specific compounds, this research sought to provide insights into the optimal extraction strategy for maximizing yield and preserving bioactive constituents.

Although these plants are widely studied, *Origanum* spp. and *Mentha* spp. were intentionally selected due to their well-established and extensively characterized essential oil composition. Their standardized and well-documented phytochemical profiles make them reliable model systems, allowing for a more accurate and comparative evaluation of extraction techniques and enhancing the credibility of the obtained results.

MATERIALS AND METHODS

Plant material

The samples were collected from the plant cultivated in Anamorava, Gjilan city in the southeastern part of Kosova with the coordinates 21°29’49.2” E 42°27’21.6” N (see Figure 1). The plant material was gathered during the vegetation period of August 2022, after the flowering stage. To preserve morphological traits, the plants were air dried, packed in paper bags, and kept in a dark and cold place (Clery, 2006; Jenner, 2006, Ibraliu *et al*., 2020; Yousaf *et al*., 2021).

Chemicals and reagents

The reagents and standards used in this study (*n*-hexane, toluene, anhydrous sodium sulfate,

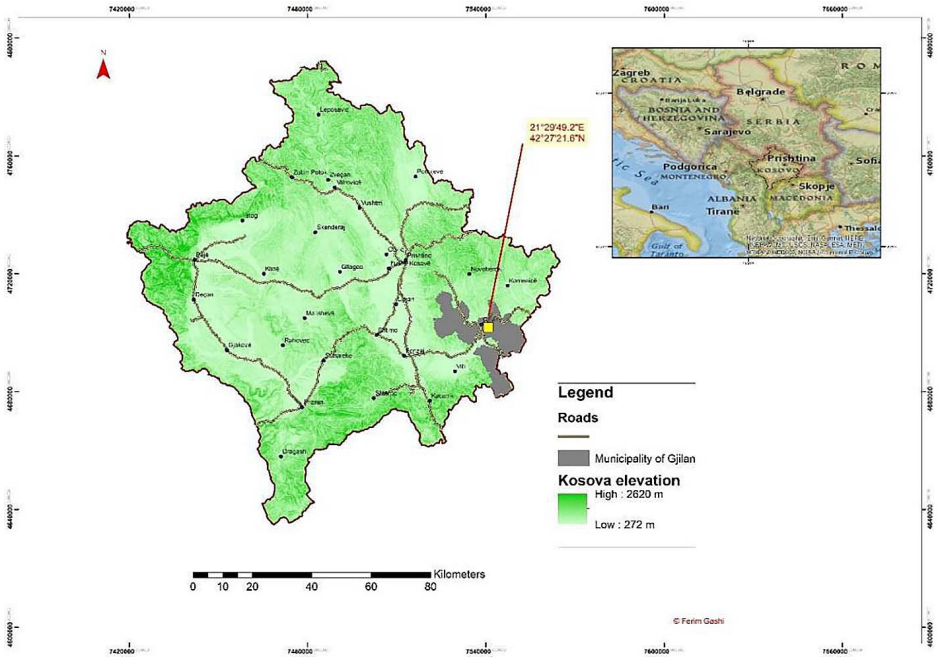

Figure 1. Sampling stations of oregano (*Origanum* spp.) and mint (*Mentha* spp.) plants in Anamorava, Gjilan city Kosova (2022)

and a standard mixture of *n*-alkanes C8-C20) were of analytical grade, purchased from Sigma Aldrich (Germany), and used without further purification. The use of high-purity reagents ensures reproducibility as well as minimizes contamination during extraction and analysis (Manjarrez-Quintero *et al*., 2024).

Extraction methods

The essential oils (EOs) of oregano (*Origanum* spp.) and mint (*Mentha* spp.) were obtained by using the HD, SC-CO_2, UAE, and HS methods. For each method, two different "captured" solvents were used: hexane and toluene. The samples were stored in dark vials in the refrigerator at 4 °C until analysis.

Essential oil isolation by hydrodistillation

To isolate the essential oil, the dried plant material (50 g of the aerial parts of the plant) was cut into smaller pieces and used for hydrodistillation for 4 h with the Clevenger-type apparatus, following the procedure of the Pharmacopeia Europe, 2007 (Ph. Eur., 2007). Hydrodistillation is a conventional technique widely used for isolating volatile compounds from aromatic plants, although prolonged heating may lead to degradation of heat-sensitive constituents (Yousaf *et al*., 2021). To collect the essential oil, 2 ml of solvent (toluene or hexane) was used. The extract was dewatered by adding 1 g of anhydrous sodium sulfate.

Essential oil isolation by supercritical carbon dioxide (SC-CO_2)

A total of 30 g of ground aerial parts of *plants* were extracted using the *Super C apparatus* (OCO Labs) with supercritical CO_2 at 150 bar and 32.5 °C for 45 min. After CO_2 evaporation, the resulting extract was collected using 2 ml of solvent (hexane or toluene) for extract collection. This method is considered an efficient and environmentally friendly technique, as it allows selective extraction of compounds while preserving thermolabile molecules due to moderate operating conditions (Herzyk *et al*., 2024).

Essential oil isolation by ultrasound-assisted extraction

The UAE process was carried out in an ultrasonic bath (ISOLAB Laborgeräte GmbH) with a working frequency of 35 kHz and a temperature of 40 °C. Milled material (1 g of plant) was mixed with 10 ml of each solvent (hexane or toluene) and placed in 20 ml amber glass vials and extracted for 30 minutes. The liquid extract was filtered

through Whatman filter paper no. 1. UAE enhances extraction efficiency through cavitation, which disrupts plant cell walls and improves mass transfer, leading to higher yields and reduced extraction time (Özdemir *et al*., 2025).

Headspace solid phase microextraction (HS/SPME)

The SPME vials (10 ml) were filled with 5 g of the aerial parts of the plant. Each vial was sealed with a screw cap equipped with a Teflon-lined septum suitable for SPME analysis. A manual SPME syringe fitted with a 100 µm polydimethylsiloxane (PDMS) fiber was placed in through the septum into the headspace above the sample. The sealed vials were placed in a water bath for 40 min at a temperature of 50 °C, to allow adsorption of the volatile compounds. After the extraction, the fiber was immediately added to the injection port of the GC and desorbed at 280 °C for 20 sec. Separation of the volatile compounds was performed on a Zebron ZB-5MSi capillary column (30 m × 0.25 mm i.d. × 0.25 µm film thickness; Phenomenex, Torrance, CA, USA). The HS techniques are particularly useful for analyzing aroma compounds and volatile fractions while avoiding sample alteration (Burzynski-Chang *et al*., 2018).

GC-FID and GC-MS analysis

The chemical composition of essential oils (EOs) obtained from oregano (*Origanum* spp.) and mint (*Mentha* spp.) by hydrodistillation (HD), ultrasound assisted extraction (UAE), headspace solid phase microextraction (HS/SPME), and supercritical CO_2 extraction (SC-CO_2) was analyzed using gas chromatography equipped with flame ionization detection (GC-FID) and gas chromatography mass spectrometry (GC-MS).

Quantitative analysis was performed using a Varian 450-GC gas chromatograph equipped with a programmed temperature vaporizing (PTV) injector and a flame ionization detector (FID). The injector and detector temperatures were set at 280 °C and 300 °C, respectively. Nitrogen was used as the carrier gas at a constant flow rate of 1.0 ml/min, and as the make-up gas at 25 ml/min. Hydrogen and air were supplied as flame gases at flow rates of 30 ml/min and 300 ml/min, respectively. Chromatographic separation was carried out on a VF-1ms capillary column (30 m × 0.33 mm i.d. × 0.25 µm film thickness). The oven temperature was initially held at 4 °C for 2 min, increased at 4 °C/min to 150°C, and then ramped at 10 °C/min to 280 °C, where it was held for 2 min. Prior to injection, 2 µl of each EO sample was diluted in n-hexane and injected in split mode (split ratio 1:50). Quantification was based on peak area integration, and results were expressed as relative percentages of the total identified compounds.

Qualitative identification of EO constituents was carried out using a Shimadzu GCMS-QP2020 system (Shimadzu, Kyoto, Japan), operated under similar chromatographic conditions as described above, except for the use of a Zebron ZB-5MSi capillary column (30 m × 0.25 mm i.d. × 0.25 µm film thickness; Phenomenex, Torrance, CA, USA), optimized for the separation of semi-volatile organic compounds. Identification of individual components was achieved by comparing their mass spectra with those in the NIST 02 and WILEY 7 N mass spectral libraries (Jennings and Shibamoto, 1980), and by comparing their experimentally determined retention indices (RI) relative to a homologous series of n-alkanes with values reported in the literature (Adams, 2015). Only the compounds with a spectral similarity index greater than 85% and consistent RI values were considered positively identified.

For the analysis of the volatile organic compounds extracted via headspace solid phase microextraction (HS-SPME), the same GC-MS system (Shimadzu GCMS-QP2020) was employed. Sample analysis followed identical conditions to those described above, ensuring comparability of data across extraction methods. As with the EO samples, compound identification in the HS-SPME extracts was based on spectral library matching and retention index confirmation.

In summary, GC-FID was used for the quantitative determination of EO composition from the HD, UAE, and SC-CO_2 extracts, while GC-MS was employed for the qualitative profiling of all samples, including headspace volatiles. This combined approach allowed for comprehensive characterization of the volatile constituents present in oregano (*Origanum* spp.) and mint (*Mentha* spp.) essential oils.

Statistical analyses

All analyses were carried out in triplicate. Values were expressed as means ± SD. The data were compared using hierarchical cluster analysis (HCA).

RESULTS

Effect of extraction techniques on chemical composition

The chemical composition of the *Mentha spp.* and *Origanum* spp. extracts varied markedly depending on the extraction method (hydrodistillation HD, supercritical CO_2 extraction SC-CO_2, ultrasound-assisted extraction UAE, and headspace solid-phase microextraction HS-SPME), as presented in Tables 1 and 2. *T*hese differences arise from variations in extraction selectivity, polarity, mass transfer mechanisms, and thermal exposure.

Across both plant matrices, HD and SC-CO_2 preferentially extracted oxygenated compounds, whereas UAE enhanced the recovery of hydrocarbon monoterpenes. HS-SPME selectively reflected volatile headspace composition rather than total extractable constituents.

DISCUSSION

Major compounds and extraction selectivity

Mentha spp.

As it is shown in Table 1, oxygenated monoterpenes dominated *Mentha* spp. extracts, particularly menthone and menthol.

HD resulted in high menthone (42.59% in toluene; 39.05% in hexane) and menthol (25.96–26.24%), confirming its effectiveness in extracting major constituents. However, prolonged heating may lead to partial degradation of thermolabile compounds.

The SC-CO_2 extraction exhibited strong selectivity toward menthol (up to 38.5%) and menthone (38.49–38.62%), while also enhancing minor compounds such as isomenthone (6.65%) and menthyl acetate (3.72%) (Table 1). This highlights its ability to preserve sensitive compounds.

In contrast, the UAE extracts showed lower oxygenated monoterpenes (27.14–32.98%) but significantly higher monocyclic monoterpenes, particularly α-phellandrene (35.65–42.18%) and limonene (24.84–25.29%), indicating a shift toward hydrocarbon compounds.

The HS-SPME analysis (Table 1) revealed a more balanced volatile profile, with menthone (38.38%) and menthol (26.11%) alongside minor esters, reflecting the headspace composition.

Origanum spp.

According to Table 2, thymol was the dominant compound across all extraction methods. The SC-CO_2 extraction yielded the highest thymol content (83.9% in toluene; 82.18% in hexane), followed by HD (61.41–63.57%) and UAE (51.1–53.15%). HS-SPME showed significantly lower thymol (23.41%), confirming its limitation to volatile fractions.

The UAE extracts (Table 2) were enriched in hydrocarbon monoterpenes, such as α-pinene (15.24–18.25%), β-pinene (up to 8.25%), and p-cymene (15.69–19.36%). HS-SPME showed dominance of α-pinene (29.06%) and camphene (8.32%), reflecting high volatility.

Distribution of chemical classes

Monocyclic monoterpenes showed notable variation between the two species and the extraction techniques. As it is presented in Tables 1 and 2, *Mentha* spp. exhibited the highest values in UAE (~60.94–67.02%), followed by SC-CO_2 (hexane) (~38.36%), while HD and HS-SPME showed considerably lower levels (~6.66–7.44%). In contrast, *Origanum* spp. demonstrated moderate levels, with the highest values observed in UAE (~15.69–19.36%) and HD (~13.46–18.18%), whereas the SC-CO_2 extracts contained negligible amounts (~0–0.25%). Overall, monocyclic monoterpenes were more abundant in *Mentha* spp., particularly when UAE was applied, while *Origanum* spp. showed a more balanced distribution across HD and UAE.

Bicyclic monoterpenes were generally present in lower amounts in *Mentha* spp. compared to *Origanum* spp. (Tables 1 and 2). In *Mentha* spp., the highest value was recorded in UAE (hexane) (~9.29%), followed by HD (~3.62–5.17%) and HS-SPME (~3.59%), while they were not detected in the SC-CO_2 extracts. Conversely, *Origanum* spp. showed significantly higher levels, with maximum values in HS-SPME (~42.03%), followed by UAE (~25.85–29.50%) and SC-CO_2 (~16.09–17.57%), whereas HD showed the lowest content (~3.82–4.97%). These findings indicate that bicyclic monoterpenes are more characteristic of *Origanum* spp., and are better recovered using the HS-SPME and UAE techniques.

Oxygenated monoterpenes were the predominant class in both species (Tables 1 and 2). *Mentha* spp. exhibited the highest values

Table 1. Detailed comparative chemical composition (%) of essential oils obtained from the aerial parts of *Mentha* spp. using different extraction techniques (hydrodistillation, supercritical CO_2 extraction, ultrasound-assisted extraction, and headspace solid-phase microextraction) in the presence of solvents with different polarity (toluene and hexane)

Compound	HD (Toluene)	HD (Hexane)	SC-CO_2 (Toluene)	SC-CO_2 (Hexane)	UAE (Toluene)	UAE (Hexane)	HS-SPME
α-Pinene	2.79± 0.20	1.5± 0.27	-	-	-	2.35± 0.27	1.23± 0.20
Camphene	0.65± 0.14	0.65± 0.07	-	-	-	3.25± 0.12	0.32± 0.06
β-Pinene	0.75± 0.18	1.47± 0.35	-	-	-	3.69± 0.11	1.46± 0.01
Sabinene	0.98± 0.05	-	-	-	-	-	0.58± 0.01
Myrcene	1.22± 0.27	0.31± 0.02	-	-	-	1.25± 0.04	0.18± 0.04
α-Phellandrene	6.21± 0.88	6.03± 0.65	4.86± 0.23	22.51± 0.68	42.18± 0.86	35.65± 0.88	5.27± 0.33
Limonene	0.31± 0.22	0.47± 0.05	2.58± 0.15	15.85± 0.23	24.84± 0.55	25.29± 0.35	0.41± 0.12
p-Cymene	0.47± 0.06	0.32± 0.01	-	-	-	-	0.29± 0.12
1,8-Cineole	0.99± 0.45	0.9± 0.20	-	14.27± 0.18	12.16± 0.33	8.52± 0.29	0.75± 0.01
γ-Terpinene	0.17± 0.07	0.47± 0.07	-	-	-	-	0.69± 0.16
cis-Sabinene hydrate	0.47± 0.09	0.67± 0.09	-	-	-	-	0.44± 0.11
Ocimene	0.15± 0.03	0.41± 0.01	-	-	-	1.25± 0.07	0.18± 0.10
Menthone	42.59± 0.91	39.05± 0.88	38.49± 1.22	38.62± 0.77	20.82± 0.77	18.62± 0.40	38.38± 0.90
Isomenthone	4.51± 0.55	3.95± 0.55	6.65± 0.77	-	-	-	4.08± 0.33
Linalool	0.39± 0.19	0.4± 0.08	-	-	-	-	1.25± 0.07
Borneol	2.87± 0.25	2.9± 0.33	4.18± 0.33	0.23± 0.04	-	-	2.96± 0.24
Menthol	25.96± 0.78	26.24± 0.45	38.5± 0.88	8.52± 0.33	-	-	26.11± 0.88
Neomenthol	0.34± 0.29	0.35± 0.04	-	-	-	-	0.39± 0.02
Terpinen-4-ol	0.24± 0.14	0.32± 0.09	-	-	-	-	0.24± 0.09
α-Terpineol	0.31± 0.07	1.17± 0.77	-	-	-	-	0.35± 0.01
Pulegone	1.28± 0.08	2.48± 0.25	-	-	-	-	1.28± 0.22
Carvone	0.22± 0.01	0.18± 0.02	-	-	-	-	0.62± 0.06
Piperitone	1.35± 0.22	0.33± 0.04	-	-	-	-	2.55± 0.29
Menthyl acetate	1.56± 0.28	2.47± 0.44	3.72± 0.25	-	-	-	2.77± 0.25
Neomenthyl acetate	0.91± 0.03	2.64± 0.15	-	-	-	-	2.93± 0.28
β-Caryophyllene	1.11± 0.44	0.51± 0.45	-	-	-	-	0.55± 0.20
α-Humulene	0.26± 0.22	0.27± 0.77	-	-	-	-	0.26± 0.03
δ-Cadinene	0.9± 0.08	1.35± 0.22	-	-	-	-	1.75± 0.15
Total (%)	**99.96**	**96.31**	**98.98**	**100**	**100**	**99.87**	**98.27**
Aliphatic compounds	1.37	0.72	-	-	-	2.50	0.36
Monocyclic monoterpenes	7.16	7.29	7.44	38.36	67.02	60.94	6.66
Bicyclic monoterpenes	5.17	3.62	-	-	-	9.29	3.59
Oxygenated monoterpenes	83.99	84.05	91.54	61.64	32.98	27.14	85.10

in SC-CO_2 (~91.54%) and HD (~84%), with similarly high levels detected by HS-SPME (~85.10%), whereas UAE showed significantly lower values (~27–33%). Similarly, *Origanum* spp. reached maximum values in SC-CO_2 (~82–84%), followed by HD (~65–73%), while UAE showed moderate levels (~51–53%) and HS-SPME considerably lower (~28.59%). This confirms that SC-CO_2 and HD are the most effective techniques for extracting oxygenated monoterpenes in both species.

Sesquiterpenes were present in very low proportions in both species (Tables 1 and 2). In *Mentha* spp., the highest values were observed

Table 2. Comparative chemical composition (%) of essential oils obtained from *Origanum* aerial parts using different extraction techniques (hydrodistillation, supercritical CO_2 extraction, ultrasound-assisted extraction, and HS-SPME) with solvents of varying polarity (toluene and hexane)

Compound	HD (Toluene)	HD (Hexane)	SC-CO_2 (Toluene)	SC-CO_2 (Hexane)	UAE (Toluene)	UAE (Hexane)	HS-SPME
α-Pinene	0.89± 0.25	1.36± 0.11	8.63± 0.76	9.36± 0.85	15.24± 0.95	18.25± 0.66	29.06± 0.75
Camphene	0.95± 0.11	0.95± 0.03	-	-	2.36± 0.22	11.25± 0.20	8.32± 0.27
β-Pinene	1.98± 0.19	2.66± 0.1.1	7.46± 0.42	8.21± 0.44	8.25± 0.39	-	4.65± 0.15
Myrcene	7.67± 0.03	6.63± 0.085	-	-	5.29± 0.45	-	6.58± 0.38
Limonene	0.82± 0.08	-	-	-	-	-	2.76± 0.13
α-Terpinene	0.86± 0.025	0.25± 0.14	-	-	-	-	0.29± 0.01
p-Cymene	11.35± 0.88	17.8± 0.28	-	0.25± 0.03	15.69± 0.85	19.36± 0.33	6.35± 0.33
γ-Terpinene	0.43± 0.17	0.13± 0.41	-	-	-	-	0.28± 0.05
Linalool	0.4± 0.22	0.14± 0.40	-	-	-	-	0.48± 0.09
Camphor	1.58± 0.33	0.16± 0.17	-	-	-	-	0.25± 0.01
Borneol	0.51± 0.45	-	-	-	-	-	0.29± 0.09
Terpinen-4-ol	1.69± 0.15	0.62± 0.29	-	-	-	-	0.36± 0.11
α-Terpineol	0.68± 0.20	0.71± 0.20	-	-	-	-	0.28± 0.18
Bornyl acetate	0.22± 0.25	-	-	-	-	-	0.48± 0.05
Carvone/Pulegone	2.66± 0.33	1.22± 0.17	-	-	-	-	0.9± 0.07
Thymol	63.57± 0.05	61.41± 0.33	83.9± 1.15	82.18± 0.1.7	53.15± 0.13	51.1± 085	23.41± 0.80
Carvacrol	1.47± 0.85	1.33± 0.55	-	-	-	-	2.14± 0.22
β-Caryophyllene	1.61± 0.27	0.53± 0.10	-	-	-	-	0.68± 0.09
α-Humulene	0.52± 0.01	-	-	-	-	-	1.2± 0.14
Total (%)	**99.86**	**95.90**	**99.99**	**100**	**99.98**	**99.96**	**88.76**
Aliphatic compounds	7.67	6.63	-	-	5.29	-	6.58
Monocyclic monoterpenes	13.46	18.18	-	0.25	15.69	19.36	9.68
Bicyclic monoterpenes	3.82	4.97	16.09	17.57	25.85	29.50	42.03
Oxygenated monoterpenes	72.78	65.59	83.90	82.18	53.15	51.10	28.59
Sesquiterpenes	2.13	0.53	-	-	-	-	1.88

in HS-SPME (~2.56%) and HD (~2.13–2.27%), while they were not detected in the SC-CO_2 and UAE extracts. A similar trend was observed in *Origanum* spp., where HD showed the highest content (~2.13%), followed by HS-SPME (~1.88%), with no detection in SC-CO_2 and UAE. Overall, sesquiterpenes represent a minor fraction and appear to be better recovered by conventional hydrodistillation and the HS-SPME methods.

Effect of solvent type (Toluene vs Hexane)

The influence of solvent type is evident in Tables 1 and 2. Hexane generally enhanced the extraction of nonpolar hydrocarbon monoterpenes (e.g., limonene, α-pinene), whereas toluene slightly favored oxygenated compounds.

In the SC-CO_2 extraction, solvent effects were less pronounced; however, hexane improved the recovery of certain hydrocarbons, confirming the role of co-solvents in modifying extraction selectivity.

Comparative analysis between *Mentha* spp. and *Origanum* spp.

Clear differences were observed between the two plant species. *Mentha spp.* (Table 1) was dominated by menthone and menthol, while *Origanum* spp. (Table 2) was characterized by thymol rich profiles.

The SC-CO_2 extraction proved optimal for both species but enhanced different target compounds: menthol/menthone in *Mentha* and thymol in *Origanum*. UAE favored hydrocarbon

monoterpenes in both cases, although compound dominance differed (α-phellandrene vs p-cymene).

The HS-SPME results revealed that *Mentha* spp. retained a balanced oxygenated volatile profile, whereas the *Origanum spp.* headspace was dominated by lighter hydrocarbons.

Comparison with literature

The present findings are consistent with the previous studies investigating extraction techniques for aromatic plants.

Hydrodistillation has been widely reported to yield high amounts of menthol and menthone in *Mentha* spp*.,* although thermal degradation may occur (Yousaf *et al*., 2021). Similarly, the SC-CO_2 extraction has been shown to enhance oxygenated monoterpenes due to its tunable solvating power and low thermal impact (Herrero *et al*., 2015; Allawzi *et al*., 2019).

For *Origanum spp.*, the dominance of thymol observed in this study aligns with the reports indicating that SC-CO_2 achieves thymol contents exceeding 80%, significantly higher than conventional methods (Allawzi *et al*., 2019). UAE has also been reported to favor hydrocarbon monoterpenes, such as p-cymene and α-pinene, due to cavitation effects (Warad *et al*., 2018).

The HS-SPME results are in agreement with Ge *et al*., (2022), who demonstrated that this technique reflects volatile headspace composition, rather than total extract yield, often underrepresenting heavier phenolic compounds like thymol.

Overall, the agreement with the literature confirms the reliability of the obtained results while highlighting the importance of method selection in phytochemical analysis.

Extraction method comparison and practical implications

The present study highlighted the critical role of extraction method selection in determining the chemical composition and quality of the *Mentha* spp. and *Origanum* spp. extracts. Each technique exhibited unique selectivity and efficiency depending on the target compounds, thermal sensitivity, and volatility.

Supercritical CO_2 extraction consistently produced the extracts with the highest purity of oxygenated and phenolic monoterpenes, such as menthol and menthone in *Mentha* spp. (Table 1) and thymol in *Origanum* spp. (Table 2). Its mild operating conditions and tunable solvating power through co-solvent use minimize thermal degradation, preserve thermolabile bioactive compounds, and provide a clear advantage for pharmaceutical, nutraceutical, and high-value essential oil applications.

Hydrodistillation effectively recovered the major oxygenated monoterpenes and phenolic compounds (Tables 1 and 2), often achieving the yields comparable to SC-CO_2. However, the prolonged heating inherent to HD can result in partial degradation of sensitive compounds, reducing extract quality for the applications requiring maximal bioactivity. Nevertheless, HD remains a practical, cost-effective technique for large-scale essential oil production.

Ultrasound-assisted extraction demonstrated distinct selectivity toward hydrocarbon-rich fractions, including monocyclic and bicyclic monoterpenes such as α-phellandrene, limonene (Tables 1), α-pinene, and p-cymene (Tables 2). The cavitation effect improves mass transfer and disrupts plant tissues, facilitating faster extraction with lower energy input. UAE is therefore highly suitable for flavor- or aroma-focused extracts and for applications where hydrocarbon monoterpenes are desired.

Headspace solid-phase microextraction captures the volatile headspace fraction, rather than the total extractable compounds, providing an accurate representation of the natural aroma profile (Tables 1 and 2). While highly informative for sensory analysis, it underrepresents heavier oxygenated compounds such as menthol or thymol, making it less suitable for bulk bioactive compound production. HS-SPME is particularly valuable for quality control and profiling of aroma-active constituents.

A comparative evaluation of the four methods underscores their complementary roles. SC-CO_2 is optimal for high-purity bioactive compounds, HD is reliable for general extraction, but may induce thermal degradation, UAE excels in hydrocarbon-rich and aroma-enhanced extracts, and HS-SPME is ideal for volatile profiling. Integrating these methods strategically can provide a comprehensive extraction approach, enabling simultaneous recovery of both bioactive and volatile fractions while maximizing the overall extract quality.

From an ecological engineering perspective, the selection of extraction techniques plays a pivotal role in reducing the environmental footprint of essential oil production processes. Green

extraction methods, such as supercritical CO_2 and ultrasound-assisted extraction, demonstrate significant advantages in terms of lower energy consumption, reduced solvent usage, and minimized generation of hazardous residues compared to conventional approaches. The ability of SC-CO_2 to operate under recyclable and non-toxic conditions, combined with the energy-efficient mechanism of UAE, aligns with the principles of sustainable process design and cleaner production. Therefore, the integration of these green technologies into industrial extraction systems represents a crucial step toward environmentally responsible utilization of plant resources, supporting the transition toward more sustainable and circular bioeconomy models.

Overall, these findings emphasize that extraction strategies should be tailored to the specific goals of the study or industrial application. The combination of modern "green" techniques, such as SC-CO_2 and UAE with traditional methods and analytical approaches allows for optimized recovery, characterization, and utilization of plant-derived compounds, in agreement with the trends reported in recent literature (Chemat *et al*., 2012; Taherpour *et al*., 2017; Putnik *et al*., 2018; Chemat *et al*., 2020; Yousaf *et al*., 2021).

Comparative hierarchical cluster analysis of extraction techniques and solvent effects on the chemical composition of *Mentha* spp. and *Origanum* spp.

Hierarchical cluster analysis (HCA), based on Euclidean distance and the UPGMA algorithm, reveals a strong correlation between extraction techniques and the chemical composition of both *Mentha* spp. and *Origanum* spp., grouping the methods into three main clusters that reflect their selectivity toward different terpene classes (Figures 2 and 3).

For *Mentha* spp., hydrodistillation, Hexane/Toluene) and HS-SPME are grouped within the same cluster, exhibiting very low dissimilarity (below 10). This indicates that hydrodistillation preserves a chemical profile closely comparable to headspace analysis, with a pronounced dominance of oxygenated monoterpenes (~84–85%). In contrast, UAE forms a completely isolated cluster with the highest dissimilarity (above 70), highlighting a significant shift in extraction selectivity toward monocyclic monoterpenes, which reach up to 67.02% (UAE-Toluene). Supercritical Fluid extraction occupies an intermediate position, where the use of toluene results in the highest purity of oxygenated monoterpenes (91.54%), while hexane enhances the extraction of monocyclic fractions. These findings suggest that HD and HS-SPME are suitable for compositional profiling, whereas UAE and SC-CO_2 enable more targeted extraction of specific metabolite groups.

A comparable clustering pattern is observed for *Origanum* spp., although with notable differences in method grouping (Figure 3). UAE (Hexane/Toluene) and HS-SPME form a cluster characterized by low dissimilarity and a strong affinity toward bicyclic monoterpenes, with HS-SPME showing the highest proportion (up to 42.03%). In this case, SC-CO_2 appears as a clearly distinct and isolated cluster, reflecting its high selectivity toward oxygenated monoterpenes (>82%) and the absence of sesquiterpenes and aliphatic compounds, which emphasizes its ability to produce chemically pure extracts. Meanwhile, hydrodistillation represents the most divergent cluster, regardless of solvent type, due to increased levels of monocyclic monoterpenes (13.46–18.18%) and aliphatic compounds. This

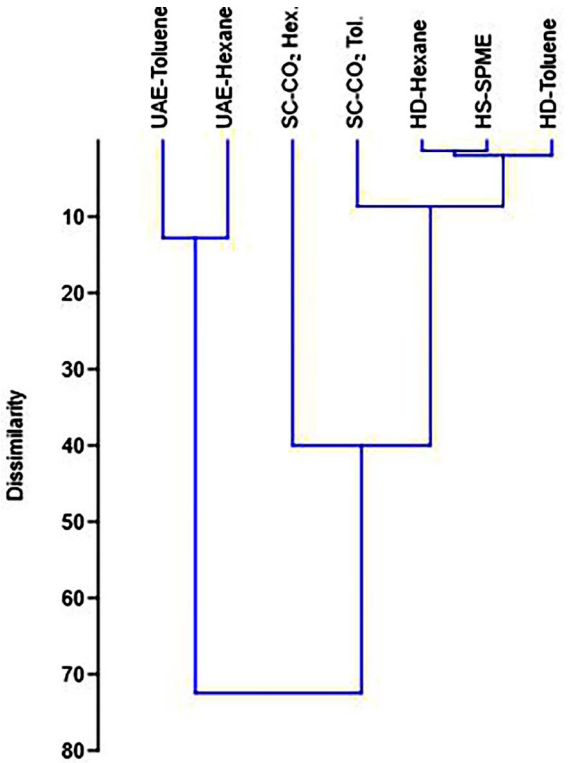

Figure 2. Dendrogram of the hierarchical cluster analysis for the extraction methods of *Mentha* spp. essential oil based on Euclidean distance and the UPGMA algorithm

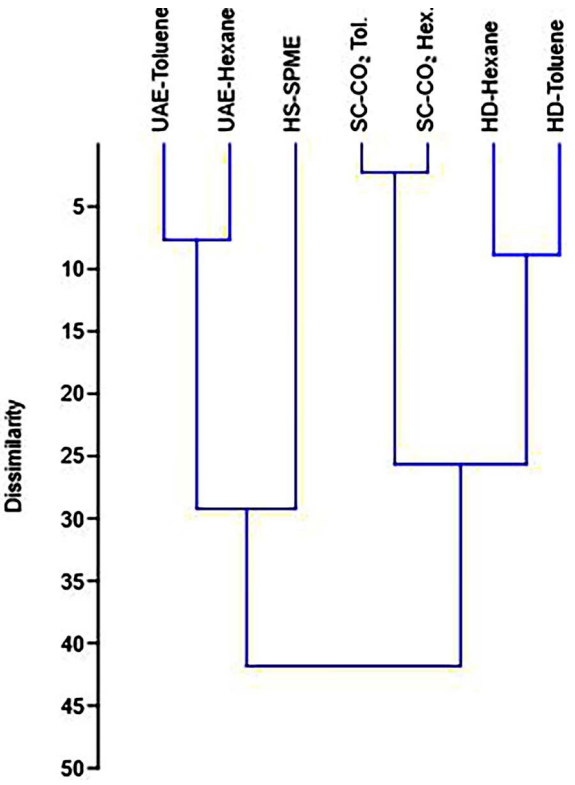

Figure 3. Dendrogram of the hierarchical cluster analysis for the extraction methods of Origanum essential oil based on Euclidean distance and the UPGMA algorithm

divergence is likely attributed to thermal effects and prolonged extraction time, which promote the chemical transformations not observed in UAE or SC-CO_2.

Overall, these results highlight that while certain techniques yield comparable chemical profiles across both plant species, others introduce marked differences in selectivity, underscoring the importance of method choice for both analytical and industrial applications.

CONCLUSIONS

This study demonstrated that the extraction technique plays a decisive role in determining the chemical composition and selectivity of compounds in *Mentha* spp. and *Origanum* spp. Significant differences were observed among hydrodistillation, supercritical CO_2 extraction, ultrasound assisted extraction, and headspace solid phase microextraction, confirming that no single method is universally optimal for all compound classes.

The SC-CO_2 extraction proved to be the most effective technique for obtaining high-purity oxygenated and phenolic monoterpenes, particularly menthol and menthone in *Mentha* spp. and thymol in *Origanum* spp., due to its mild conditions and tunable selectivity. HD showed comparable efficiency in recovering major compounds, although prolonged thermal exposure may affect sensitive constituents. In contrast, UAE favored the extraction of hydrocarbon monoterpenes, resulting in the extracts enriched with compounds such as α-phellandrene, limonene, α-pinene, and p-cymene. HS-SPME provided valuable insight into the volatile headspace composition, although it underrepresented less volatile and heavier compounds.

Comparative analysis between the two species revealed distinct phytochemical profiles, with *Mentha* spp. dominated by menthone and menthol, and *Origanum* spp. characterized by thymol-rich extracts. Additionally, solvent type influenced extraction selectivity, with hexane enhancing nonpolar compounds and toluene favoring slightly more polar constituents.

Overall, the findings highlight the importance of selecting an appropriate extraction method based on the target compounds and intended application. The complementary nature of the applied techniques suggests that their combined use can provide a more comprehensive characterization and efficient recovery of bioactive and aromatic compounds from plant materials.

From a green engineering and sustainability perspective, the results of this study underline the necessity of transitioning toward environmentally responsible extraction strategies in the valorization of aromatic and medicinal plants. The demonstrated efficiency of supercritical CO_2 and ultrasound-assisted extraction highlights their potential to reduce energy consumption, minimize solvent use, and lower environmental impact compared to conventional techniques. Integrating such green technologies into industrial-scale processes can significantly contribute to cleaner production systems, improved resource efficiency, and reduced ecological footprint. Consequently, this study supports the development of sustainable extraction frameworks aligned with the principles of ecological engineering and circular bioeconomy, promoting the efficient and responsible utilization of natural resources.

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