Research Article

Region Specific Volatile Composition of *Mentha aquatica* Essential Oil: Insights

Ali Sabah Alhasan

Department of Horticulture Sciences and Landscape, Agricultural College, University of Al-Qadisiyah, Al-Diwaniyah, Iraq.

Abstract | Mint *Mentha aquatica* L. also known as water mint belongs to 'Lamiaceae' family, is a perennial aromatic plant naturally grown in various regions of Iraq and has been utilized in traditional medicine for its diverse therapeutic properties since prehistoric times. The current experiment focused on exploring the chemical constituents present in essential oil (EO) extracted from naturally grown *M. aquatica* and to evaluate its potential for further pharmacological applications. Fresh aerial plant parts were collected, followed by washing and oil extraction through hydrodistillation using a Clevenger-type apparatus to obtain the essential oil. The extracted oil was analyzed by gas chromatography mass spectrometry (GC-MS) to identify its volatile constituents. Around 44 bioactive compounds were identified representing the major chemical profile of the essential oil. The results depicted that major components detected were caryophyllene (5.15%), followed by isocarveol (4.90%), menthofuran (4.59%), menthone (4.52%), and eucalyptol (3.53%). These compounds are well known for various biological activities, suggesting the therapeutic applications of extracted oil. The results highlight the chemical variation of *M. aquatica* essential oil and suggest that environmental and geographical factors may influence its profiling. It is suggested that phytochemical exploration of water mint from different Iraqi regions to complete characterization is necessary for the assessment of its potential in pharmaceutical and industrial usage.

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***Correspondence** | Ali Sabah Alhasan, Department of Horticulture Sciences and Landscape, Agricultural College, University of Al-Qadisiyah, Al-Diwaniyah, Iraq; **Email**: ali.alhasan@qu.edu.iq
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Introduction

Plant based natural products have gained prime importance across the globe, which motivating the researchers to explore bioactive compounds present in aromatic and medicinal plants particularly those belonging to *Lamiaceae* family (Uritu *et al*., 2018). Among other aromatic plants, *Mentha aquatica* L., commonly also known as water mint, holds significant value for pharmacological and cosmetic applications. Mint herb thrives best in wet and marshy environmental conditions and is distributed across the globe i.e. Europe, North Africa and Western Asia, including Iraq. Although, mint has been widely explored for its aromatic profile and therapeutic uses, some species remains underexplored in certain regions, where its phytochemical variability and potential uses are yet to be systematically explored (Benabdallah *et al*., 2016; Pires *et al*., 2024).

Essential oils (EOs) are basically complex mixtures of volatile secondary metabolites, that serve as chemical defense mechanisms in plants against biotic and abiotic stress, are also key contributors to their biological activities in plants (Siddiqui *et al*., 2024; Ben-Miri, 2025). These essential oils are basically composed of monoterpenes, sesquiterpenes, and their oxygenated derivatives which differ notably among all species, environments and chemotypes. *Mentha* essential oil has been comprehensively explored and is well-known to possess a wide array of bioactivities including antimicrobial, antioxidant, anti-inflammatory, insecticidal and cytotoxic properties (Chizzola, 2013; Mimica-Dukic and Bozin, 2008). These characteristics insure widespread application of *Mentha* oils in traditional medicine, pharmaceuticals, cosmetics and food preservation. Moreover, the therapeutic potential of *Mentha aquatic* still attain limited attention by researchers as compared to other species of mint including *Mentha piperita* and *Mentha arvensis* (Mimica-Dukić *et al*., 2003; Salehi *et al*., 2018).

In Iraq, *Mentha aquatica* grown naturally in several regions but still it has not been the gain attention for detailed phytochemical or pharmacognostic studies. The ecological conditions of Iraq ranging from arid to wetland habitats create diversified microenvironment that can affect the biosynthesis and accumulation of essential oil constituents which indirectly effect the quality of essential oil (Al-Bayati, 2009; Feng *et al*., 2021). Several, experiments have been conducted to assess the chemical profile of essential oils is influenced by genetic factors, environmental stress, soil composition, altitude, climate and seasonal changes. These factors can affect significantly, qualitative and quantitative variations in volatile compounds present in essential oil even within the same species (Qian *et al*., 2024). From the previous studies we can conclude that region-specific characterization has become essential, both for understanding the adaptive biology of the plant and for detecting potentially novel or abundant bioactive constituents (Luo *et al*., 2024).

Gas chromatography mass spectrometry (GC-MS) is well-known effective technique for the detailed analysis of volatile organic compounds in plant extracts. This technique offers highly sensitive, reproducible and compound resolution, making it a necessary tool for chemotaxonomic experiments and quality control of essential oil (Yan *et al*., 2023). However, there is no doubt about use of GC-MS in characterization of plant EOs globally; data on the chemical composition of *M. aquatica* from Iraq remain limited leaving a gap in both scientific knowledge and practical application (Sharmeen *et al*., 2021).

The current study was planned to explore the essential oil composition of *Mentha aquatica* naturally growing in Iraq a region that is characterized by semi-arid conditions interspersed with seasonal wetlands. The mint essential oil was extracted through hydrodistillation and analyzed through GC-MS for detection and quantification of volatile compounds. By providing a comprehensive chemical composition of this specie from an underreported region, this study aims to contribute authentic data for future pharmacological, ecological and industrial investigation. Moreover, these results may serve as a foundation for the sustainable and efficient utilization and conservation of native medicinal flora in Iraq.

Materials and Methods

Collection and preparation of plant material

Wild-growing specimens of *Mentha aquatica* were collected from natural wetlands in Al-Muthanna Governorate, Iraq, during the spring flowering period. The aerial parts mainly the leaves and tender stems were harvested early in the day to preserve volatile constituents. The plant material was authenticated by a botanical expert, and a representative voucher specimen was preserved in the departmental herbarium for future reference. Post-harvest, the plant samples were rinsed with clean distilled water to eliminate surface impurities. They were then air-dried under shaded, ambient conditions (25–30°C) for 6–8 days. After drying, the material was coarsely ground using a stainless steel cutter and stored in airtight containers until oil extraction.

Essential oil extraction

The essential oil was isolated using hydrodistillation with a Clevenger type apparatus. A total of 100 grams of dried plant material was mixed with 500 mL of distilled water in a round-bottom flask and subjected to hydrodistillation for three hours. The distillate was collected, and the oil layer was separated from the aqueous phase. To remove residual moisture, the oil was treated with anhydrous sodium sulfate and filtered through Whatman No. 1 filter paper. The final oil sample was stored in sealed amber glass vials at 4°C to protect it from light and degradation prior to analysis (Ben Hassine *et al*., 2021).

GC-MS analysis

Chemical profiling of the essential oil was performed using a Shimadzu GC-MS QP 2010 system. The instrument was fitted with an Rtx-5MS capillary column (30 meters in length, 0.25 mm internal diameter, and 0.25 µm film thickness). The mobile phase consisted of high-purity helium, used as the carrier gas at a steady flow rate of 1.0 mL/min (Singh *et al*., 2020). A 1 µL aliquot of the essential oil, diluted 1:10 with n-hexane, was injected in split mode (split ratio 1:50). The injector temperature was maintained at 250°C. The oven temperature was initially set at 60°C for two minutes, then ramped up by 3°C per minute until reaching 250°C, where it was held for 10 minutes. Mass spectral data were obtained in Electron Ionization (EI) mode at 70 eV. The scanning range for mass detection was set between 50 and 550 m/z. The ion source temperature was 230°C, and the interface temperature was 280°C.

Identification of constituents

The chemical constituents of the essential oil were identified by comparing the mass spectra of the peaks with those in the NIST and Wiley libraries provided with the instrument software. Additionally, retention indices were determined using a homologous series of *n*-alkanes (C8–C24), injected under identical chromatographic conditions. Final identifications were confirmed by evaluating both spectral matches and calculated retention indices against established literature data (Salim *et al*., 2025).

Results and Discussion

Gas chromatography–mass spectrometry (GC-MS) analysis of *Mentha aquatica* essential oil extracted from samples collected in Al-Muthanna Governorate led to the detection of 44 distinct volatile constituents, collectively accounting for 99.9% of the total oil composition (Table 1). This high percentage reflects the analytical robustness of the extraction and detection procedures used. The gas chromatogram (Figure 2) illustrates a well-resolved profile, demonstrating the chemical complexity of the essential oil and the efficiency of the DP-5MS capillary column used during analysis.

GC-MS Analysis of Water Mint (*Mentha aquatica*) Essential Oil

Figure 1: *Mentha aquatica grown wildly in the Al-Diwaniyah region of Al-Qadisiyah (Iraq).*

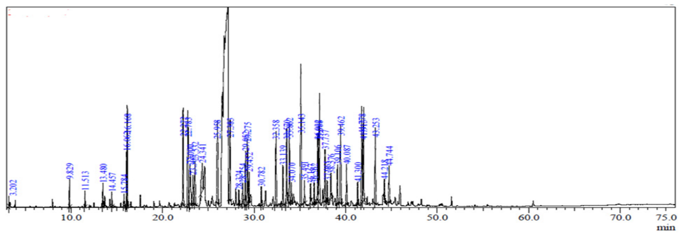

Figure 2: *Mass spectrum of unknown compound in Mentha aquatica essential oil.*

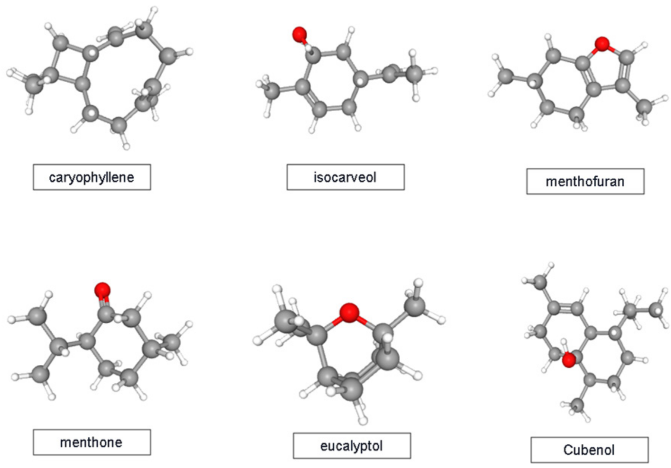

Figure 3: *The structures of the major chemical compounds identified in the Mentha aquatica essential oil.*

Among the identified compounds, caryophyllene was the dominant constituent (5.15%), followed closely by isocarveol (4.90%), menthofuran (4.59%), menthone (4.52%), and eucalyptol (3.53%) (Figure 3).

Table 1: *Chemical compositions of the Mentha aquatica essential oil.*

Pic	RT	%	Compounds
1	3.2	0.18	β-Methylbutanal
2	9.8	0.85	2,5-Diethyltetrahydrofuran
3	11.5	0.49	α-Pinene
4	13.5	0.78	β-Pinene
5	14.5	0.43	3-Octanol
6	15.8	0.39	o-Cymene
7	16.1	2.12	Isolimonene
8	16.2	3.53	Eucalyptol
9	22.3	4.10	cis-3-Heptadecene
10	22.8	4.52	Menthone
11	23.0	3.11	Thanisol
12	23.3	1.27	Dihydrocarvone
13	23.5	2.06	(-)-4-Terpineol
14	24.3	2.42	α-Terpieol
15	26.0	4.90	Isocarveol
16	27.4	3.20	Piperitone oxide
17	28.3	0.51	cis-Carvone oxide
18	28.8	0.71	Anethole
19	29.1	2.37	Isolongifolan-7-α-ol
20	29.3	3.23	D-Verbenone
21	29.5	1.26	Diosphenol
22	30.8	0.74	Isopulegol acetate
23	32.4	4.17	Carveol acetate
24	33.1	1.74	Copaene
25	33.6	3.37	D-Germacrene
26	33.9	3.65	β-Elemene
27	34.1	0.81	Grindelene
28	35.1	5.15	Caryophyllene
29	35.5	0.86	β-Cubebene
30	36.2	0.68	cis-β-Copaene
31	36.6	0.85	α-Caryophyllene
32	36.9	3.23	trans-β-Copaene
33	37.1	4.19	(E)-4-Chloro-2,3-dimethyl-1,3-hexadiene
34	37.7	2.53	β-Yalangene
35	38.0	1.07	β-Selinene
36	38.4	1.42	β-Humulene
37	39.1	1.67	α-Copaene
38	39.5	4.59	menthofuran
39	40.1	1.79	Isoledene
40	41.8	4.12	(+)-Spathulenol
41	41.9	3.91	Cariophyllene epoxide
42	43.3	3.55	Cubenol
43	44.2	0.86	δ-Cadinene
44	44.7	1.62	α-Cadinol
Total		99%	

These constituents are known for their bioactive properties, particularly in antimicrobial, anti-inflammatory, and antioxidant contexts, which highlights the potential pharmacological value of the oil. Caryophyllene, a naturally occurring sesquiterpene, is well-known for its for its potential of anti-inflammatory and analgesic properties. Likewise, the monoterpenoids, menthofuran and menthone are prominent associated with antispasmodic and gastrointestinal uses.

Detailed analysis in previous studies conducted in many other regions discloses notable chemical variation. For instance essential oil extracted from *Mentha aquatica* grown in Algeria, was mainly composed of bergamiol, α-pinene, geranyl butyrate, α-terpineol and linalool (Chaker *et al*., 2014). Contradiction to this, Esmaeili *et al*. (2006) reported that oil extracted from Iranian *Mentha aquatica* was rich in linalyl acetate (26.1%), α-pinene (22.7%) and linalool (13.8%). In another study conducted in Iran on peppermint depicted that cineole, menthofuran and (E)-caryophyllene were predominant chemical constituents in essential oil (Hassanpouraghdam *et al*., 2022). Above discussed results support the hypothesis that different geographical origin plays a key role in shaping the volatile profile of mint species.

Significant diversity is evident across different species of genus menthe, for example, *Mentha spicata* grown in Iran displayed significant concentrations of carvone (64.30–7.45%) and limonene (24.21–6.59%) (Rasekh *et al*., 2023). This variation in vital components within species and geographic locations highlight the effect of genetic background and environmental interactions on essential oil biosynthesis pathways. Several factors may involve that can create variation in the composition of essential oil. Environmental factors like temperature, exposure of light, humidity and soil characteristics can affect secondary metabolite quantity in essential oil. Additionally, harvesting stage, altitude and photoperiod also have effect on volatile compound concentration. Diversity in ecotypes, developmental stage of plant and plant part used further contribute to variation in essential oil composition (Lahlou *et al*., 2024; Zorpeykar *et al*., 2022; Velička *et al*., 2022).

In the current study, the relatively lower concentration of menthol related compounds and dominance of caryophyllene and isocarveol proposes a unique chemotype of *Mentha aquatica* adapted to the semi-arid conditions in south of Iraq. This highlighting ecological adaptability and potential for yielding region specific bioactive compounds of species. Such variation is not only of creates attraction for pharmacological but may also for industrial usage in the formulation of fragrances, flavorings and therapeutic products.

Collectively these results highlight the necessity for region specific characterization of essential oils, particularly from underexplored regions. The characteristic chemical fingerprint of *Mentha aquatica* naturally grown in Al-Muthanna contributes vital data to the phytochemical landscape of the genus and supports further exploration into its ecological adaptations and possible commercial applications.

Conclusions and Recommendations

The present investigation explored the chemical composition of essential oil derived from *Mentha aquatica* collected in Al-Muthanna, Iraq. Around 44 volatile compounds were detected through GC-MS analysis, in the essential oil extracted. The most abundance of vital constituents as follows caryophyllene, menthofuran, isocarveol, menthone and eucalyptol. Chemical profile indicates that particular environmental conditions at collection site and differentiates Iraqi *M. aquatica* from those reported in other countries in earlier studies. The variation observed in composition of essential oil underlines the adoptability of plant to local climatic conditions and suggests that geographical factors significantly affect the biosynthesis pathway of its volatile compounds. These results depicted the potential importance of this plant as a source of natural compounds for different uses, including in therapeutic, food preservation and other applications. Current work contributes original valuable information on a species that has been underrepresented in phytochemical analysis within Iraq. Further experiments are encouraged for seasonal assessment, ecological and biological influences on essential oil yield and composition, as well as to explore its biological activities. Such efforts would help sustainable application and preservation of Iraq's local medicinal plants.

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Novelty Statement

This study presents the first detailed GC-MS analysis of *Mentha aquatica* essential oil from the Al-Muthanna region of Iraq. The identification of a unique chemotype, dominated by caryophyllene and isocarveol, distinguishes it from previously reported profiles in other regions. These findings contribute novel insights into the phytochemical diversity and regional adaptation of *M. aquatica*.

Generative AI or AI-assisted Technology Statement
The author(s) declare that no Genrative AI was used in the creation of this manuscript.

Conflict of interest
The authors have declared no conflict of interest.

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