Chemo-geographical variations in volatile profiles and antifungal properties of essential oils from Iranian *Mentha longifolia* L. populations

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Abstract

Mentha longifolia L. is one of the most important species of the Lamiaceae family, consisting of seven varieties in Iranica flora. This herb has been used as a traditional medicine for a long time. There are many studies on the identification and correlation between this species and environmental factors. The first part of this research was designed for determinate the essential oil (EO) composition of *M. longifolia* growing wild in the north-west of Iran. A total of twenty compounds have been identified, accounting for 92.82–100% of the total oil composition through the High Performance Liquid Chromatography (HPLC) method. Results have shown that the major components of the oil and their percentages were pulegone (7.18-52.23%), menthone (10.18-32.54%), and piperitenone oxide (0.77-16.01%). In addition, the essential oil showed strong levels of antifungal activity against the tested microorganisms: *Botrytis cinerea, Penicillium* sp., and *Rhizopus stolonifera*. At a similarity of 50%, the essential oil properties were divided into 2 sub-clusters, including cluster I, which contained mainly 6 ecotypes, and cluster II, which included 2 ecotypes. In addition, minimal inhibitory concentration for fungi growth revealed 2 main clusters consisting of 3 and 4 ecotype groups.

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Key words: mint, phytochemical, Azerbaijan provinces, fungi, pulegone.

Contributions: NM and VA, conceptualization, methodology, formal analysis and investigation; NM, VA, writing-original draft preparation; NM, VA, VZN and AM; writing - review and editing; VA, VZN and AM, resources, supervision; all authors have read and approved the manuscript.

Funding: this study was not financially supported by an organisation or other.

Conflicts of interest: the authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Availability of data and materials: the datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Received: 6 December 2024.
Accepted: 15 March 2025.
Early view: 16 July 2025

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Licensee PAGEPress, Italy
Journal of Biological Research 2025; 98:13456
doi:10.4081/jbr.2025.13456

Introduction

Lamiaceae (Labiatae) is an important plant family because of its medicinal properties due to its large amounts of flavonoids, phenolic acids and essential oils. This family is also known for its aromatic members and includes more than 7,200 species and 240 genera.[1] The genus *Mentha* consists of about 25-30 species; most of them are found in wet places such as rivers and they grow across the temperate regions of the world. They are widely distributed in North America, central and southern Europe, South Africa, southwest Asia and Australia.[2] *Mentha longifolia* L., commonly known as horsemint, is a wild and perennial herb that can grow up to 2-meter height. Several biological activities have been found for some species of *Mentha*, such as antibacterial and antifungal.[3] *M. longifolia* syrup was reported to be a safe and effective option for supporting menstrual health and maintaining regular bleeding in women's menstrual disorders.[4] Historically, mint plants have been used because of their medicinal properties and much research has focused on the identification of secondary metabolites in these plants. They are rich in phenolic compounds and other substances with antioxidant activity. Several flavonoids and phenolic compounds have been identified in mint plants.[5]

After the development of modern chromatographic systems, rapid and convenient methods can be used for the standardization of herbal drugs based on their natural compounds. Terpenoids are the most common and structurally diverse natural products found in many plants with more than 20,000 known members that are important due to their wide industrial application such as flavoring agents, perfumes and insecticides agents.[6]

Various species of mint have been used in traditional medicine for the treatment of bronchitis, flatulence, anorexia and liver complaints, because of their anti-inflammatory, carminative, antiemetic, diaphoretic, antispasmodic, and anticatharral activities.[7,8] Based on our knowledge, there are no reports on *M. longifolia* essential oil in Iran. So, in this study, the main purpose is to analyze phytochemical

and antifungal properties of wild *M. longifolia* in different regions of Iran. Furthermore, another aim of this research is the determination and identification of chemical compounds in this plant using High Performance Liquid Chromatography (HPLC) method.

Materials and Methods

Aerial parts (leaves and head branches) of *M. longifolia* were collected from 8 regions of Azerbaijan provinces in Iran in September 2017 (Table 1). The plants were identified by specialists of the herbarium of Tabriz University in Iran. The plant material was dried first, grinded into fine power, and then stored in plastic bags at 5°C. Overall, 8 ecotypes were collected and labeled from A_1 to A_8.

Chemical profiling of *M. longifolia* ecotypes

In this study, 100 g of dried aerial parts of *M. longifolia* powder was hydro-distilled for 3h using a Clevenger apparatus (Shenzhen Haocheng Instrument Co., Ltd., Shenzhen, China). The supernatant (essential oil) was collected and dried over anhydrous sodium sulfate and kept in the dark at 4°C until Gas Chromatography–Mass Spectrometry (GC-MS) analysis and other uses (antifungal assay).

GC-MS profile of essential oil Compounds was determined through a gas chromatography system (6890-5973) coupled to mass spectrometer equipped with a HP-5MS column (60 m length, 0.22 mm inner diameter and 0.25 µm film thickness; Agilent Technologies, Santa Clara, California, USA). The most volatile chemical compounds of the essential oil were identified by comparison between their retention indices (KI) for n-alkanes (C_6-C_{24}).

The HPLC system was KNAUER device (KNAUER Wissenschaftliche Geräte GmbH, Berlin, Germany) consisting of variable wavelength Ultra Violet (UV) detector. The analytical column was Kromasil C_{18} column (Kromasil, AkzoNobel, Bohus, Sweden). The mobile phase was methanol and phosphate buffer.

Table 1. Characteristics of the sampling sites and composition of the essential oil in the eight ecotypes of *M. longifolia*.

Sample	Location	Height (m)	Slope	Latitude	Longitude	Temp (°C)	Relative humidity (%)
A_1	West Azerbaijan-Khoy	964	NE	38 51 23	45 12 45	12.62	58.5
A_2	West Azerbaijan- Oroumieh	1465	SW	37 17 41	45 07 49	11.9	61.16
A_3	West Azerbaijan-Sardasht	1283	Flat	36 8 24	45 30 59	14.57	50.16
A_4	East Azerbaijan-Maragheh	1496	SW	37 25 22	46 14 44	15	50.33
A_5	East Azerbaijan-Oskoo	2083	W	37 49 34	46 16 06	12.47	52.16
A_6	East Azerbaijan-Bostan abad	1911	NE	37 43 48	46 54 44	7.6	61.48
A_7	West Azerbaijan-Salmas	1452	N	38 14 24	44 49 38	11.6	58.08
A_8	East Azerbaijan-Azershahr	2123	NE	37 43 25	46 11 53	15.2	52.28

No	Compound		Composition (%)							
		KI*	A_1	A_2	A_3	A_4	A_5	A_6	A7	A_8
1	Pulegone	1243	52.23	29.73	30.76	34.7	20.12	7.18	7.96	48.55
2	Iso-Pulegone(sic)	1163.32	7.81	-	-	-	-	-	-	7.52
3	Myrcene	981.91	1.49	-	-	-	-	-	-	-
4	ß-pinen	979.47	1.67	1.77	-	-	-	-	-	3.17
5	Sabinen	971.74	0.87	0.57	-	-	-	2.3	-	-
6	α pinen	937.6	1.01	0.54	-	-	-	-	-	1.23
7	Menthone	1146.18	14.96	28.64	28.37	22.01	32.54	14.5	10.18	21.55
8	1,8-cineol	1029.01	8.07	7.81	-	-	5.16	3.2	6.91	8.07
9	Caryophyllen oxide	1589.58	4.88	2.18	-	4.21	3.45	-	-	2.51
10	Menthol	1168.74	-	8.53	6.01	3.53	3.93	4.5	5.98	-
11	Spatholenol	1548	-	0.67	-	-	-	3.8	-	-
12	Piperitenon	1338.9	-	1.48	-	-	-	-	-	0.88
13	Piperitenon oxide	1354.78	0.77	4.44	15.74	10.04	3.51	9.02	16.01	1.14
14	Menthil acetate	1321	-	0.82	-	-	1.53	-	-	-
15	ß-caryophyllen	1433.2	-	1.92	6.59	7.86	-	2.17	4.22	-
16	Iso-menthyl acetate	1307.54	-	1.47	-	-	-	1.5	1.66	-
17	Piperiton	1249.9	-	-	12.53	14.72	-	-	-	-
18	Dhydrocarvon(trans)	984.34	-	-	-	1.2	15.65	-	-	-
19	Pipriton epoxide	1253.75	-	8.46	-	-	3.71	51.83	46.46	
20	Iso-D-hydrocarvoeol	1583.73	-	-	-	-	1.36	-	-	-
21	Total (%)		93.76	99.03	100	98.27	99.79	92.82	99.38	94.62

KI, Kovats index.

Flow rate was 0.5 mL/min. The effluent was monitored for UV absorption at 214 nm; 10 µL of sample was injected and all separations were performed at ambient temperature.[9]

Determination of flavonoids

The flavonoids content was determined as described by Krizek *et al*.[10] Leaf samples were homogenized in a mortar and pestle with 3 mL 1% acetic acid-ethanol solvent (1:99, v/v). The homogenate was distributed into centrifuge cups and centrifuged at 18,000 g for 30 min. After that, the supernatant was incubated in a water bath for 10 min at 80°C and let it cool down at room temperature. After cooling, the amounts of flavonoids were determined at 300 nm absorbance using a UV-Vis spectrophotometer (Shimadzu Corporation, Kyoto, Japan). Flavonoid content was expressed as µmol/g Fresh Weight (FW) and the concentration of flavonoids was calculated using an extinction coefficient of flavonoids $\varepsilon = 33\ 000\ mol^{-2}cm^{-1}$.

Determination of total phenolic content in the essential oils

Folin-Ciocalteu's method was used to determine total phenol in leaf extract. Briefly, 0.5 mL of extract and 7 mL deionized water were added to a test tube and then 0.5 mL of Folin Reagent were transferred to the solution. After 3 minutes, 1 mL of saturated sodium carbonate solution was added to the sample and the solution volume was increased to 10 mL by adding distilled water. After 1 hour, the absorbance was measured at 725 nm using a Cary 50 UV-Vis spectrophotometer (Agilent Technologies, Santa Clara, USA). The total phenolic content of the extract was measured using a standard curve based on mg of caffeic acid per gram of extract.[11]

Antifungal testing

Three fungal species (*Penicillium* sp., *Botrytis cinerea* and *Rhizopus stolonifer*) were used in this study. Fungi were collected from the mycology collection of Science and Research Branch, Islamic Azad University, Tehran, Iran. Essential oils extracted from *M. longifolia* were solved in tween 80 solution; the emulsion was prepared and then mixed with Potato Dextrose Agar medium (PDA). Stock cultures were maintained on PDA. After autoclaving, the PDA medium was cooled to 42-45°C before being poured into Petri dishes. Then, concentrations of 250, 500, 800, 1000 and 1300 mg/L essential oil for *Botrytis cinerea*, 250, 500, 800, 1000, 1300 mg/L essential oil for *Penicillium sp*. and 1000, 2500, 3000, 3500 mg/L essential oil for *Rhizopus stolonifer* were added to the PDA medium and stirring was applied to create a uniform emulsion which was subsequently allowed to solidify. After that, seven-day-old culture fungi isolates (5 mm disc) were inoculated in the center of the media plates. Fungal growth inhibition was determined as the diameter of inhibition zones around the discs. All samples were tested 3 times. Subsequently, Petri dishes were incubated at 25°C for 1-14 days. The difference in incubation times for each strain (1 day for *R. stolonifer*, 3 days for *B. cinerea*, and 14 days for *Penicillium* sp.) is primarily due to the varying growth rates and metabolic characteristics of each fungal species. Each species responds differently to environmental conditions like temperature, humidity, and nutrient availability, which influences the time required for observable growth. After incubation, the increase of inhibition zone diameters was measured and noted. Minimum Inhibitory Concentration (MIC) was defined as the lowest essential oil concentration that prevents microorganism's growth. The MIC of different concentrations of essential oil was determined by the Abbot formula.[11]

Statistical analysys

Experiments were performed 3 times and all data was analyzed by SPSS 23.0.0.0 (completely randomized design for phytochemical experiments and factorial design for fungal experiments). The outcomes are reported as average ± SD of the triplicates ($p \leq 0.05$).

Results

Chemical profiling of *M. longifolia* ecotypes

Based on the results, 97.21% of essential oil of *M. longifolia* is composed by 20 active compounds. The obtained essential oil yield was 0.17% (A_1) - 0.52% (A_2) (v/w) (Table 1). All samples included pulegone, piperitenone oxide, menthone as main components. Other components consisted of piperiton epoxide, piperiton, dhydrocarvon (trans), iso-pulegone (sic), 1,8-cineol, menthol, ß-caryophyllen, caryophyllen oxide, spatholenol, ß-pinen, sabinen, myrcene, isomenthyl acetate, iso-D-hydrocarvoeol, menthyl acetate, pinen.

Flavonoid's content

In our study, flavonoids' content ranged from 0.5 µg/mL (ecotype A1) to 4.5 µg/mL (ecotype A3) (Table 2).

Table 2. Phytochemical properties of *M. longifolia*; mean±standard deviation (n=3)

population	Carotenoids (mg g^{-1})	Flavonoid (mg mol g^{-1} FW)	Total phenolic (mg caffeic acid g^{-1} fresh weight)
A_1	^{cd}0.003±0.0000	^{e}0.5±0.05	^{d}1.5±0.01
A_2	^{e}0.001±0.0001	^{d}0.9±0.02	^{c}11±0.26
A_3	^{de}0.002±0.0000	^{a}4.5±0.05	^{b}27.27±4.98
A_4	^{bc}0.004±0.0000	^{c}1.21±0.15	^{a}75.52±8.79
A_5	^{cd}0.003±0.0001	^{d}0.8±0.01	^{d}3.3±0.03
A_6	^{de}0.002±0.0000	^{b}3.01±0.16	^{c}11.74±1.57
A_7	^{a}0.006±0.0001	^{c}1.07±0.06	^{d}1.2±0.01
A_8	^{ab}0.005±0.0024	^{c}1.1±0.02	^{d}4.09±0.00

Means within each column followed by the same letter are not different according to the Least Significant Difference (LSD) test ($p \leq 0.05$).

Total phenolic content

Total phenolic content of the tested *M. longifolia* ecotypes ranged from 1.54 (A_1) to 75.52 (A_4) mg caffeic acid/g FW (Table 2).

Antifungal testing

In the current study, antifungal activity of essential oil has been investigated against 3 fungi strains. As shown in Table 3 and Figure 1 the essential oil showed strong levels of antifungal activity against the tested microorganisms. The presence of pulegone, menthone and piperitenone epoxide can be responsible for antifungal activity. Based on the results, A_6, A_7 and A_2 ecotypes have the best antimicrobial activity which may be associated with relatively high menthone, piperitenon oxide and piperitenone epoxide contents. Table 1 also indicates that these ecotypes live at the lowest average temperature and highest relative humidity in comparison with others. Menthone, piperitenone oxide and carvone also showed substantial antimicrobial activities.

Dendrogram of similarities

Figure 2 illustrates the similarities among eight populations of the genus *Mentha* for cluster analysis of essential oil characteristics. The results revealed the formation of two groups. Cluster I primarily included ecotypes A1, A2, A7, and A8, which exhibited similar antifungal properties. Cluster II consisted of ecotypes A3, A4, A5, and A6. The differences between these two groups may be attributed to the lower essential oil content and the higher concentrations of pulegone and iso-pulegone in the essential oil extracted from ecotypes A1 and A8 compared to the other ecotypes. The presence of ecotypes 2 and 7 alongside ecotypes 1 and 8 suggests that the mere presence of pulegone and iso-pulegone does not solely account for the antifungal activity, indicating that other bioactive compounds in the essential oil also contribute to the antifungal effect.

Discussion

Previous studies indicated that the yield of essential oil extracted from of *M. longifolia* was: 0.39%, 1.39-4.05%, 0.9-1.8% and 1.55-

Table 3. Minimal inhibitory concentration (MIC) of *M. longifolia* essential oil on the three studied fungi.

Fungi	MIC (ppm)							
	A_1	A_2	A_3	A_4	A_5	A_6	A_7	A_8
Botrytis cinerea	800	800	1000	1000	1000	1000	800	800
Penicillium sp.	1300	1300	1300	1300	1300	1000	1000	1300
Rhizopus stolonifer	3500	3000	3000	3000	3000	3000	3500	3000

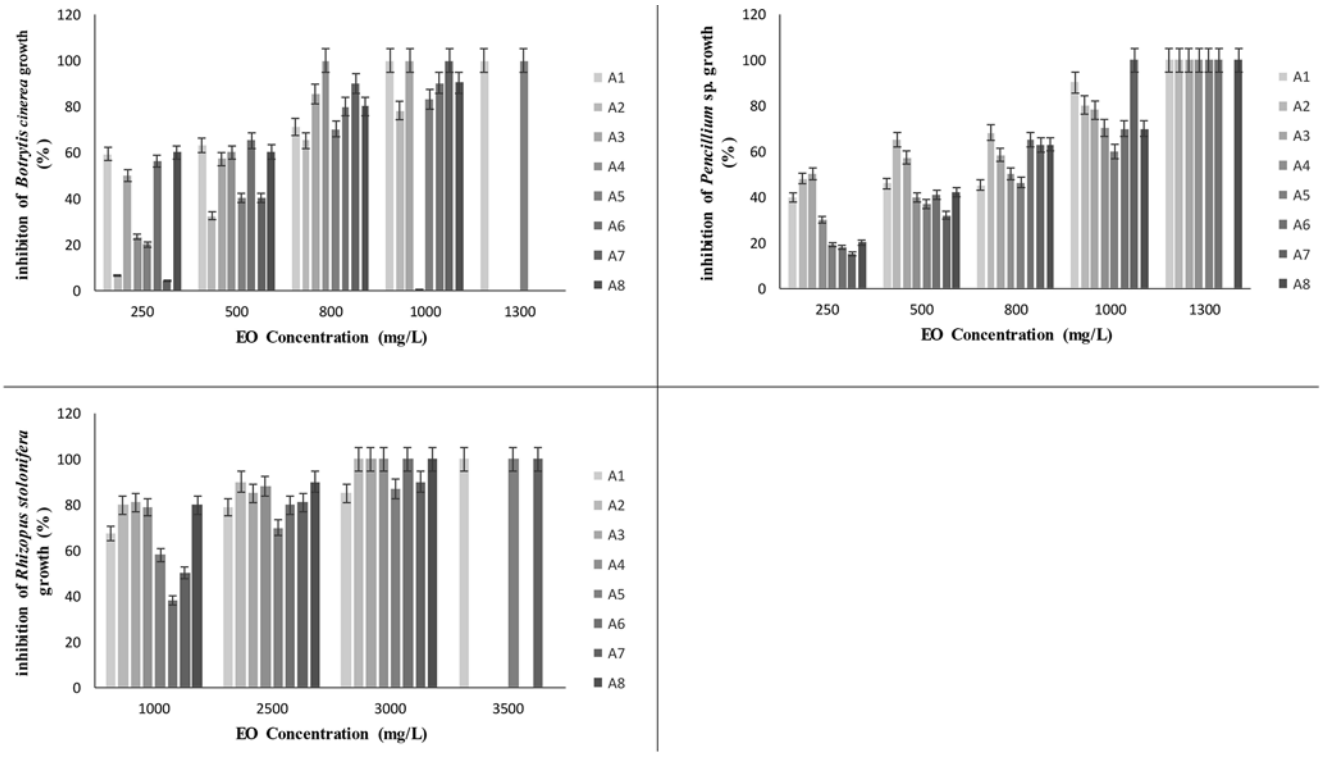

Figure 1. Percent inhibition of fungi *Botrytis cinerea*, *Pencillium* sp., *Rhizopus stolonifera* growth at different *M. longifolia* essential oil (EO) concentrations; mean ± standard deviation (SD).

1.64%.[12] The discrepancy between the results can be explained by differences in environmental conditions of the target regions that had effects on the essential oil contents of *M. longifolia.*

El Hadji *et al*.[13] indicated that the major compounds identified in *M. longifolia* dried plants were pulegone (42.4%), menthone (21.2%), 1,8-cineole (11.4%) and iso-menthone (13.2%). In another study, it was shown that 97.8% of the total oil composition was made up of the following components: Pulegone (32.3%), Isopulegone (8.9%), Menthone (13.8%), and 1,8-Cineole (8%).[14] Previous studies also reported that the major compounds among 23 identified compounds were piperitenone oxide, pulegone and 1,8–Cineole. Based on other studies, the major components of the oil and their percentage were *cis*-piperitone epoxide (7.8-77.6%), piperitenone oxide (1.5-49.1%), carvone (0.0-21.5%), pulegone (0.3-5.4%), menthone (0.0-16.6%), thymol (1.5-4.2%).[15] The results of a phytochemical study showed that pulegone with 31.54% is the most active ingredient of plant.[16] In contrast with this study, the main composition of *M. longifolia* L. native to Ilam is beta-Phellandrene.[17]

Studies have identified compounds like menthone, piperitone oxide, cis-piperitone epoxide, and menthol in the essential oils of this plant. For instance, research from Saudi Arabia reported that the essential oil of *M. longifolia* contains menthone, carvone, menthol, piperitone oxide, pulegone, piperitenone, d-limonene, and 1,8-cineole as dominant components.[18] Similarly, a study from Senegal found that the essential oil of *M. longifolia* includes compounds such as 1,8-cineole, menthone, is-omenthone, and pulegone.[19]

Previous studies showed that *M. longifolia* essential oil is the main source of piperitone, pulegone, mihydrocarvone, cis-dihydrocarvone and piperitenone. These studies also pointed out that ecological aspects such as climatic and soil conditions have a strong impact on the essential oil content.[7] Based on this study, genetic variation, growth stages, parts of plant utilized and maturity variation are important factors that determine the composition and the yield of *M. longifolia* essential oil. The differences between essential oil content and composition of the collected *M. longifolia* leaves can be explained by environmental and geographical factors (temperature, rainfall, altitude, etc.). Consequently, our finding has shown that the chemical composition of the essential oil obtained from the leaves and flowers of *M. longifolia* collected from eight different regions in Iran have different qualitative and quantitative properties.

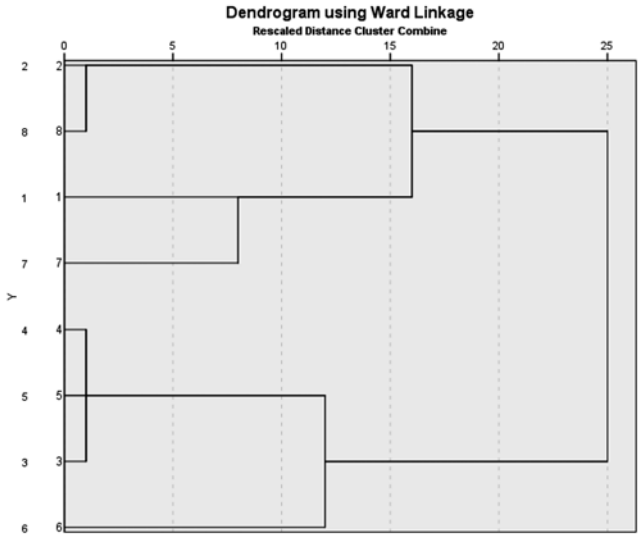

Figure 2. Dendrogram of minimal inhibitory concentration using Ward's minimum variance method of cluster analysis (1, West Azerbaijan-Khoy; 2, West Azerbaijan-Oroumieh; 3, West Azerbaijan-Sardasht; 4, East Azerbaijan-Maragheh; 5, East Azerbaijan-Oskoo; 6, East Azerbaijan-Bostanabad; 7, West Azerbaijan-Salmas; 8, East Azerbaijan-Azershahr).

The flavonoids content of *M. longifolia* varies across different studies. Stanisavljević *et al*.[20] reported a content of 6.52 mg/g FW. Bahadori *et al*.[21] reported that the total flavonoid content of ethanol extracts of *M. longifolia* was 23.68 ± 0.20 mg rutin equivalents (RE) /g.

Zaidi and Dahiya[22] showed *Mentha piperita* has the highest contents of total phenolics (12.63± 0.878 µg gallic acid equivalents - GAE) followed by *Mentha spicata* (9.41 ± 0.594 µg GAE). Another study indicated the amount of 67.05±0.85 GAE/g, 30.327±1.4 g GAE/100 g and 6.08 mg RE /g FW for the total phenolic content.[21] Phenolic compounds can contribute to the quality and nutritional value in terms of modifying color, taste, aroma, and flavor. These compounds also have health beneficial effects. Phenolic compounds also play a main role in plant defense mechanisms to counteract Reactive Oxygen Species (ROS) to survive and prevent molecular, and by microorganisms, insects, and herbivores damages. High concentration of phenolic and flavonoid compounds in *M. longifolia* expresses strong potential health benefits. The previous studies also showed the presence of high phenolic and flavonoid content in *M. longifolia* and other *Mentha* species.[23]

Zaidi and Dahiya[22] reported that the essential oils from mint species *Mentha spicata* and *Mentha piperita* have antimicrobial activity against *Aspergillus* spp. and *Candida albicans*, so that they can be used as natural antimicrobial agents. The inhibitory effect of spearmint oil against *Escherichia coli, Bacillus subtilis, Aspergillus niger* and *Candida albicans* was also studied by Sulieman *et al*.[24] and Yazgi *et al*.[25] who reported that ethanol and methanol extracts of *M. longifolia* plant inhibited the growth of fungi *Fusarium moniliforme* and *Alternaria citri* at low concentrations (0.04 g/mL). Gulluce *et al.*[26] studied on methanol extract from the aerial parts of *M. longifolia* subsp. *longifolia* and did not find any antimicrobial activity; but the essential oil of *M. longifolia* has phytotoxic effect on seedling growth of *Avena fatua* due to the main components such as menthol, menthone and carvone. Also, *M. piperita* and *M. spicata* showed strong ability as antifungal agent against the plant pathogenic fungi.[27] In another study on the acetone extract of *M. longifolia,* good antifungal activity against the fungal strains *i.e.*, *Aspergillus fumigatus* and *Geotrichum candidum* was observed.[28]

Essential oil of *M. longifolia* exhibited fungicidal activity against *Aspergillus* and *Fusarium* species at a concentration of 10 µL/mL.[29] Antifungal activity on *Trichophyton menthagrophytes* and yeast *Candida albicans* was observed at a lower dilution concentration.[7] Previous studies reported that essential oil of *M. longifolia* has higher antimicrobial and antifungal activity than the tested commercial substances.[30] The findings indicated that the hydrogel formulation containing essential oil exhibited the most substantial activity against the majority of the tested phytopathogens in a dose-dependent manner.[31]

Fungal diseases annually impose irreparable damage worldwide; the essential oil of *M. piperita* has been evaluated for its antimicrobial properties, showing promising results against bacteria and fungi responsible for food spoilage. This suggests its applicability as a natural preservative in the food industry.[32] Variations in the chemical compositions of essential oil might be due to the varied climatic conditions of the regions, isolation regimes, and adaptive metabolism of plants. Environmental conditions are other important

factors affecting the plant growth and components yield. For instance, research reported that light and day length affected plants essential oil contents. In a study on rosemary (*Rosmarinus officinalis*), the application of end-of-day red and far-red light treatments significantly affected both the content and composition of essential oils compared to control plants.[33] Other studies have also reported the influence of mineral nutrients, drought, light intensity and altitude on plants growth and essential oil contents.[34]

The increasing resistance of pathogens to antibiotics and the undesirable side effects associated with their use have led to the exploration of alternative treatments, such as essential oils from various herbs. *Mentha* essential oils, for instance, have demonstrated significant antibacterial properties. A study on *Mentha* × *piperita* (peppermint) essential oil revealed its potential microbicidal activity against common phytopathogens, suggesting its use as a natural antimicrobial agent.[35] Additionally, research has shown that essential oils from *Mentha* species exhibit antibacterial effects against different strains of *Staphylococcus aureus*, including Methicillin-Resistant *Staphylococcus aureus* (MRSA).[36] In line with our study essential oil derived from oregano showed the highest effect against all tested bacteria and fungi.[37] These findings support the traditional use of herbal essential oils in treating various infectious diseases.

In the modern era, various pharmacological activities have been confirmed for *M. longifolia.* The differences in the antimicrobial activities may be due to different geographical location, age of the plant, different extraction methods of essential oil, cultivar types and seasonality.[38]

Conclusions

This study aims to evaluate the essential oil composition of *M. longifolia*, which grows wild in northwestern Iran. The analysis of compound variations across different ecotypes from eight distinct regions demonstrated that environmental conditions play a significant role in the quality and quantity of these compounds. The results identified 20 chemical constituents, accounting for 92.82% to 100% of the total essential oil composition. The primary components and their respective concentrations were pulegone, menthone, and piperitenone oxide.

The present study indicates that *Mentha longifolia* essential oil can serve as a potential source of natural antimicrobial compounds, attributed to its phenolic constituents and strong antioxidant potential. Additionally, the results reveal promising antifungal properties. Examination of the antifungal effects of essential oils from the eight studied regions showed that the essential oils of ecotypes A2, A6, and A7 exhibited the highest antifungal activity, which may be attributed to the presence of bioactive compounds such as menthol and pulegone. Further research is required for the identification of biologically active compounds, characterization, and purification of the crude extracts of *Mentha* species.

References

1. Bräuchler C, Meimberg H, Heubl G. Molecular phylogeny of Menthinae (Lamiaceae, Nepetoideae, Mentheae)–taxonomy, biogeography and conflicts. Mol Phylogenet Evol 2012;55:501-23.
2. Vining KJ, Hummer KE, Bassil NV, et al. Crop wild relatives as germplasm resource for cultivar improvement in mint (*Mentha* L.). Front Plant Sci 2020;11:1217.
3. Yousefian S, Esmaeili F, Lohrasebi T. A comprehensive review of the key characteristics of the genus *Mentha*, natural compounds, and biotechnological approaches for the production of secondary metabolites. Iran J Biotechnol 2023;21:e3605.
4. Mokaberinejad R, Zafarghandi N, Bioos S. *Mentha longifolia* syrup in secondary amenorrhea: a double-blind, placebo-controlled, randomized trial. DARU J Pharm Sci 2012;20:97.
5. Eftekhari A, Khusro A, Ahmadian E, et al. Phytochemical and nutra-pharmaceutical attributes of *Mentha* spp.: A comprehensive review. Arab J Chem 2021;14:11-3.
6. Fan M, Yuan S, Li L, et al. Application of terpenoid compounds in food and pharmaceutical products. Fermentation 2023;9:119.
7. Džamić V, Soković AM, Ristić MD, et al. Antifungal and antioxidant activity of *Mentha longifolia* (L.) Hudson (Lamiaceae) essential oil. Bot Serb 2010;34:57-61.
8. Hussain AI. Characterization and biological activities of essential oils of some species of Lamiaceae. PhD Thesis. Faculty of Sciences, University of Agriculture, Faisalabad, Pakistan; 2009.
9. Elshafie HS, Viggiani L, Mostafa MS, et al. Biological activity and chemical identification of ornithine lipid produced by *Burkholderia gladioli* pv. *agaricicola* ICMP 11096 using LC-MS and NMR analyses. J Biol Res 2018;90:6534.
10. Krizek DT, Kramer GF, Upadhyaya A. UV-B response of cucumber seedlings grown under metal halide and high-pressure sodium/deluxe lamps. Physiol Plant 1993;88:350-8.
11. Seevers P, Daly J. Studies on wheat stem rust resistance controlled at the *Sr6* locus. I. The role of phenolic compounds. Phytopathol 1970;60:1322-8.
12. Abbaszadeh B, Valadabadi SA, Farahani HA, et al. Studying of essential oil variations in leaves of *Mentha* species. Afr J Plant Sci 2009;3:217-21.
13. El Hadji T, Dienaba SS, Boubacar, et al. Guide maraîchage au Sénégal: carotte, oignon, haricot vert, tomate, chou, pomme de terre et bissap. [Market gardening guide in Senegal: carrot, onion, green bean, tomato, cabbage, potato, and hibiscus] Cirad-Agritrop 2015; available from: https://agritrop.cirad.fr/580006/
14. Ertaş A, Gören A, Haşimi N, et al. Phytochemical and biological investigations on *Mentha longifolia* subsp. *noena*. Planta Med 2012;78:PI123.
15. Sharopov FS, Sulaimonova VA, Setzer WN. Essential oil composition of *Mentha longifolia* from wild populations growing in Tajikistan. J Med Act Plants 2012;1:76-84.
16. Mahmodi R, Tajik H, Farshid A, et al. Phytochemical properties of *Mentha longifolia* L. essential oil and its antimicrobial effects on *Staphylococcus aureus*. Armaghane Danesh Bimonth J 2011;16:400-12.
17. Mardani S, Nasri H, Rafieian-Kopaei M, et al. Herbal medicine and diabetic kidney disease. J Nephropharmacol 2013;2:1-2.
18. Anwar F, Alkharfy KM, Rehman NU, et al. Chemo-geographical variations in the composition of volatiles and the biological attributes of *Mentha longifolia* (L.) essential oils from Saudi Arabia. Int J Pharmacol 2017;13:408-24.
19. Diop SM, Guèye MT, Ndiaye I, et al. Chemical composition of essential oils and floral waters of *Mentha longifolia* (L.) Huds. from Senegal. Am J Essent Oils Nat Prod 2016;4:46-9.
20. Stanisavljević D, Đorđević S, Zlatković B, et al. Antimicrobial and antioxidant activity of the essential oils obtained from *Mentha longifolia* L. Hudson, dried by three different techniques. Rec Nat Prod 2014;8:61-5.
21. Bahadori MB, Zengin G, Bahadori S, et al. Phenolic composition and functional properties of wild mint (*Mentha longifolia* var. *calliantha* (Stapf) Briq.). Int J Food Prop 2018;21:183-93.
22. Zaidi S, Dahiya P. *In vitro* antimicrobial activity, phytochemical analysis and total phenolic content of essential oil from *Mentha*

spicata and *Mentha piperita*. Int Food Res J 2015;22:2440.
23. Elansary HO, Mahmoud EA. Egyptian herbal tea infusions' antioxidants and their antiproliferative and cytotoxic activities against cancer cells. Nat Prod Res 2015;29:474-9.
24. Sulieman AME, Abdelrahman SE, Rahim AMA. Phytochemical analysis of local *Mentha spicata* leaves and detection of the antimicrobial activity of its oil. J Microbiol Res 2011;1:1-4.
25. Yazgi M, Awad D, Jreikous B. Screening of the antifungal activity of plant *Mentha longifolia* crude extracts against two fungi *Alternaria citri* and *Fusarium moniliforme*. J Entomol Zool Stud 2015;3:359-64.
26. Gulluce M, Sahin F, Sokmen M, et al. Antimicrobial and antioxidant properties of the essential oils and methanol extract from *Mentha longifolia* L. ssp. *longifolia*. Food Chem 2007;103:1449-56.
27. Kostik V, Gjorgjeska B, Petkovska S. *Mentha* L. essential oils composition and *in vitro* antifungal activity. IOSR J Pharm 2015;5:1-7.
28. Hosny M. Phenolic constituents and the biological activity of *Mentha longifolia*. Nat Prod Res 2014;50:103-16.
29. Mimica-Dukić N, Božin B, Soković M, et al. Antimicrobial and antioxidant activities of three *Mentha* species essential oils. Planta Med 2003;69:413-19.
30. Elshafie HS, Nuzzaci M, Logozzo G, et al. Biological investigations on the role of hydrogel formulations containing bioactive natural agents against some common phytopathogens of *Phaseolus vulgaris* L. and seed germination. J Biol Res 2020;93:9219.
31. Mkaddem M, Bouajila J, Ennajar M, et al. Chemical composition and antimicrobial and antioxidant activities of *Mentha* (*longifolia* L. and *viridis*) essential oils. J Food Sci 2009;74:M358-63.
32. Voirin B, Brun N, Bayet C. Effects of daylength on the monoterpene composition of leaves of *Mentha* × *piperita*. Phytochem 1990;29:749-55.
33. Mulas G, Gardner Z, Craker LE. Effect of light quality on growth and essential oil composition in rosemary. Acta Hortic 2006;723:427-32.
34. Ardakani M, Mafakheri S. Designing a sustainable agroecosystem for wheat (*Triticum aestivum* L.) production. J Appl Environ Biol Sci 2011;1:401-13.
35. Camele I, Gruľová D, Elshafie HS. Chemical composition and antimicrobial properties of *Mentha* × *piperita* cv. 'Kristinka' essential oil. Plants 2021;10:1567.
36. Horváth P, Koščová J. *In vitro* antibacterial activity of *Mentha* essential oils against *Staphylococcus aureus*. Folia Vet 2017;61:71-7.
37. Elshafie HS, Mang SM, Camele I. Blended formulations of oregano-sage essential oils: antimicrobial, phytotoxic, and anti-quorum sensing investigations. J Biol Res 2024;97:11999.
38. Mitscher LA, Drake S, Gollapudi SR. A modern look at folkloric use of anti-infective agents. J Nat Prod 1987;50:1025-40.