

Chemical Diversity and Chemotypic Variation of *Mentha spicata* Essential Oils from Different Regions of Iran

Research paper

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Abstract **Aim:** This study investigated the chemical diversity of essential oils (EOs) extracted from eight *Mentha spicata* samples collected across different regions of Iran.

Material and Methods: Essential oils obtained by hydrodistillation were analyzed using gas chromatography mass spectrometry (GC-MS), and their compositional patterns were examined through principal component analysis (PCA).

Results: Essential oil yields ranged from 0.3% to 1.6% (v/w), consistent with previously reported values for Iranian mints. Oxygenated monoterpenes were the dominant constituents, with carvone, D-limonene, and 1,8-cineole as major compounds. PCA explained 94.47% of the total variance and revealed clear clustering of samples, indicating significant chemical differentiation among populations. Three chemotypic patterns were identified: (1) a carvone-limonene (spearmint-type) profile found in most samples, (2) a mixed carvone/carvone oxide type, and (3) a 1,8-cineole-rich type.

Conclusion: The chemical variation observed among Iranian *M. spicata* populations may result from both genetic diversity and environmental influences on essential oil biosynthesis. These findings expand the understanding of Iranian *M. spicata* chemotypic diversity and provide a basis for selecting desirable chemotypes for pharmaceutical, aromatic, and flavor applications.

Keywords *Mentha spicata* – essential oils – chemical diversity – GC-MS – chemotypes

INTRODUCTION

Many contemporary pharmaceuticals are either natural compounds or derivatives of medicinal plants, which have served as crucial sources of therapeutic agents for millennia (Kinghorn et al., 2011). Among these, mints (*Mentha* spp.) are particularly notable; species containing menthol have been valued for over 2,000 years, with archaeological evidence of their presence in Egyptian tombs and references in the Bible (Brian M. Lawrence, 2006). Approximately 25 to 30 species and several hybrids are classified within the genus *Mentha* of the family Lamiaceae (Singh and Pandey, 2018). Based on phylogenetic analyses of morphology, chromosome numbers, and essential oil composition, the genus is divided into four sections: *Tubulosae* (Briq.) Tucker, *Eriodontes* Benth. in DC., *Pulegium* (Mill.) Lam. & DC., and *Mentha* L. (Brian M. Lawrence, 2006). The *Mentha* genus is distributed globally and is widely cultivated for the production of pharmaceutical and culinary products (Saqib et al., 2022).

Mints are rich in essential oils and phenolic compounds, including phenolic acids and flavonoids, which are associated with a broad spectrum of biological effects such as antimicrobial, antioxidant, and anti-inflammatory activities (Mamadaliyeva et al., 2020; Yousefian et al., 2023). *Mentha* species have traditionally been used in Iran to alleviate various ailments (Tafrihi et al., 2021). They are incorporated into traditional formulations such as oxymel (*Sekanjabin*), which contains various aromatic plants including mint combined with honey, water, and vinegar (Darani et al., 2023), as well as in national food products such as *Kashk* and *Doogh* beverages (Shahrajabian and Sun, 2023). In traditional Iranian medicine, mints and their extracts, including Araq-e-Nana (peppermint distillate), are widely used to treat nausea, diarrhea, rheumatism, bloating, liver disorders, gallstones, and other conditions. They are also frequently employed to alleviate not only digestive problems but also respiratory tract disorders, headaches, and hemorrhoids. Araq-e-Nana

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Table 1. Examined *Mentha spicata* samples from Iran: codes, locations, and GPS coordinates

Sample	Location	Coordinates (latitude/longitude)	Altitude (m)	Avg high/low °C (August)	Humidity (%)	Location description
S1	Sonqor (Kermanshah)	34.78 N/47.60 E	~1,450 m	~36°C/ ~21°C	35%–45%	Semi-arid climate with warm summers and cold winters; collected on 05.08.2024
S2	Mashhad (Razavi Khorasan)	36.30 N/59.61 E	~1,000 m	~34°C/ ~20°C	25%–35%	Temperate-dry, moderate humidity; collected on 12.08.2024
S3	Ilam (Ilam Province)	33.64 N/46.42 E	~1,350 m	~33°C/ ~20°C	30%–40%	Semi-humid mountainous western area with mild summers, collected on 07.08.2024
S4	Shaft (Gilan Province)	37.17 N/49.40 E	~80 m	~30°C/ ~24°C	70%–85%	Humid Caspian (subtropical) high annual rainfall; collected on 15.08.2024
S5	Kermanshah (Kermanshah Province)	34.33 N/47.10 E	~1,320 m	~36°C/ ~21°C	30%–40%	Semi-arid moderate continental climate, fertile soils, collected on 06.08.2024
S6	Sangar (Gilan Province)	37.18 N/49.70 E	~50 m	~30°C/ ~24°C	75%–90%	Coastal plain, humid subtropical climate, collected on 21.08.2024
S7	Iranshahr (Sistan–Baluchestan)	27.20 N/60.69 E	~600 m	~41°C/ ~29°C	15%–25%	Hot-arid desert climate with strong sunlight and minimal rainfall; collected on 25.08.2024
S8	Langrood (Gilan Province)	37.20 N/50.15 E	~5 m	~30°C/ ~25°C	75%–90%	Very humid coastal near the Caspian Sea, mild temperatures; collected on 01.09.2024

is a traditional Iranian herbal distillate prepared through the distillation process, during which mint essential oils are combined with water. In Iranian medicine, it is valued for its cooling properties (Tafrihi et al., 2021). Spearmint (*M. spicata*) is also a key component of the traditional tea mixture known as Moroccan mint tea (blend of green tea and mint), which is likewise popular in Iran (Anbri et al., 2022).

Although *Mentha* essential oils have been extensively studied in many countries, research conducted in Iran remains limited and fragmented, typically focusing on individual cultivated species without comprehensive comparative analyses (Moetamedipoor et al., 2021; Shahbazi, 2015). Our study aimed to analyze the essential oils of eight wild-growing populations of *M. spicata* L. collected from various regions of Iran.

MATERIAL AND METHODS

Plant material

Aerial parts of *Mentha* species were collected from eight distinct geographical and climatic regions of Iran (Table 1, Figure 1). The samples used in this work were cultivated mint plants grown by local farmers in their natural regional

environments. The harvesting took place between August 18 and September 10, 2024, on sunny days at the onset of the flowering stage. After collection, the plant material was shade-dried at ambient temperature. The dried leaves were separated from stems and flowers and subsequently crushed to the appropriate particle size for further analysis. Direct utilization of the leaf material enhances the overall homogeneity of the resulting sample. The collected specimens were taxonomically identified by Assoc. Prof. Dr. Silvia Bittner Fialová, a specialist in the genus *Mentha* L. Representative dried samples (Vouchers GHM1–GHM8) have been deposited at the Department of Pharmacognosy and Botany, Faculty of Pharmacy, Comenius University Bratislava, for reference purposes.

Essential oil isolation

Essential oil (EO) was isolated by hydrodistillation according to European Pharmacopoeia 11th ed. using a Clevenger apparatus. A quantity of 10.0 g of crushed leaf drug was placed in a 500-ml flask and covered with 150 ml of distilled water (R) as the distillation liquid. Distillation was carried out for 2 hours at a rate of 3 to 4 ml per minute (European Pharmacopoeia 11, 2025). The essential oil was analyzed by



Figure 1. Collection sites of eight *Mentha spicata* samples (S1–S8) across Iran. Each point corresponds to a population used for essential oil extraction and GC-MS analysis.

GC-MS immediately after distillation. The essential oils were distilled once due to the limited amount of plant material available. Yields were calculated as follows:

$$\text{Yield (\% v/w)} = \left(\frac{\text{mL essential oil}}{\text{g dry leaves}} \right) \times 100$$

Gas Chromatography–Mass Spectrometry (GC-MS) Analysis

Briefly, 100 μL of the mixture (distilled essential oil dissolved in 1 mL of n-hexane) was further diluted in 1 mL of n-hexane and transferred into 1.2 mL vials. The GC-MS system (Scienc Instruments, Goes, the Netherlands) was equipped with a GC module 8300, MD module 8700 SQ, AutoInjector 8410, and MS WorkStation software. Helium (5.0 grade) was used as the carrier gas. Separation and identification of components were performed on a SolGel-WAX capillary column (30 m $\times$ 0.25 mm $\times$ 0.25 μm ; Kinesis SGE Analytical Science, Australia). The analysis was carried out in a split mode with a split ratio of

1:10, an equilibration time of 3.8 min at 40°C, and an injection volume of 1 μL . The oven temperature program started at 60°C, increased at a rate of 8°C/min to 70°C, then at 5°C/min to 150°C, and finally at 4°C/min to 230°C, where it was held for 1 min. The total analysis time was 48.5 min. The ion source, injector, and interface temperatures were maintained at 250°C. Full-scan mass spectra were acquired in the m/z range of 50–300. Data processing, including baseline correction and compound identification, was performed using MS WorkStation 8 software (Scienc Instruments, Goes, the Netherlands). Compound contents are expressed as relative percentages (%). Only compounds present in amounts greater than 0.5% were considered. Identification of compounds was based on comparison with the NIST library, with a minimum similarity index of 90% considered as putative identification. GC-MS analyses were carried out in duplicate. The parallel runs produced identical qualitative profiles

Table 2. Essential oil yields of *Mentha* samples collected from different regions of Iran

Sample	Location	Plant material (g)	EO volume (mL)	EO yield (% v/w)
S 1	Sonqur, Kermanshah	10	0.10	1.0
S 2	Mashhad	10	0.06	0.6
S 3	Ilam	10	0.03	0.3
S 4	Shaft, Gilan	10	0.04	0.4
S 5	Kermanshah	10	0.03	0.3
S 6	Sangar, Gilan	10	0.08	0.8
S 7	Iranshahr, Sistan, and Baluchistan	5	0.08	1.6
S 8	Langrood, Gilan	5	0.06	1.2

Principal Component Analysis (PCA)

Principal component analysis (PCA) was conducted using PAST software, version 5.3 (Hammer et al., 2001), freely available at <https://www.nhm.uio.no/english/research/resources/past/>. The main components (PC 1 and PC 2) were extracted and visualized with scatter plots. Loadings plots for PC 1 and PC 2 were generated to assess the contribution of individual variables to the principal components.

RESULTS

EO yields of *M. spicata* samples varied significantly across the eight regions of Iran, ranging from 0.3% to 1.6% (v/w) (Table 2). The highest yield was observed in Sample 7 from Iranshahr, while the lowest was recorded in Samples 3 and 5.

GC-MS analysis identified a total of 35 major compounds in the essential oils, representing more than 99% of the total composition in all samples (Table 3). D-limonene and carvone were the predominant constituents in most samples, while 1,8-cineole was abundant in Sample 4. Minor constituents such as α -pinene, β -pinene, and β -myrcene were detected in variable amounts, contributing to the chemical diversity of the oils.

The essential oils of all eight spearmint samples were strongly dominated by monoterpenes, whereas sesquiterpenes occurred only in minor proportions. Across the dataset, the monoterpene fraction was primarily represented by D-limonene, 1,8-cineole, and carvone, which collectively constituted the bulk of the volatile profile in every sample. In contrast, sesquiterpenes such as caryophyllene, β -bourbonene, D-germacrene, and isogermacrene D contributed only trace to low-level amounts.

PCA was performed to assess the variation in essential oil composition among *Mentha spicata* samples collected from different regions of Iran. The first two principal components (PC 1 and PC 2) explained 94.47% of the total variance (73.45% and 21.02%, respectively). The scatter plot of PCA scores (Figure 2) showed a clear separation among samples along PC 1 and PC 2. Sample S4 was positioned far on the

positive side of PC 1. Sample S2 displayed a positive PC 2 score (Figure 2).

Samples S1, S3, S5, S6, S7, and S8 were grouped closely together, indicating a similar essential oil profile among them. The loading plots for PC 1 and PC 2 (Figures 3 and 4, respectively) revealed that 1,8-cineole had the highest positive contribution along PC 1, whereas carvone and D-limonene contributed strongly in the negative direction. Along PC 2, carvone dioxide had a strong positive loading, while D-limonene, 1,8-cineole, and carvone showed negative contributions.

DISCUSSION

The present study elucidates the considerable chemical diversity of essential oils from eight *Mentha spicata* samples collected across different regions of Iran. Hydrodistilled oils were analyzed using GC-MS, and compounds were identified through comparison of retention indices and mass spectral data. Essential oil yields are summarized in Table 2, while detailed compositional profiles are provided in Table 3. Essential oil yields ranged from 0.3% to 1.6% v/w, which fall within Iranian mints ranges; for example, yields of 0.62–1.24 mL/100 g in *M. longifolia* and 0.49–1.54 mL/100 g in *M. spicata* ecotypes have been reported. Also, another recent study reported *M. spicata* essential-oil concentrations ranging from 0.20% to 2.60% (w/w), which are in agreement with the results of the present work. (Golparvar and Hadipanah, 2016; Van Haute et al., 2023). Thus, our yields align with previous regional findings. GC-MS analysis revealed that oxygenated monoterpenes were the dominant constituents. The eight EOs resolved into three clear patterns. A detailed evaluation of the essential oil compositions revealed that all spearmint samples were dominated by monoterpenoid compounds, both hydrocarbon and oxygenated. In contrast, sesquiterpenoids formed only a minor portion of the overall profiles. The quantified monoterpenes, including hydrocarbons such as α -pinene, β -pinene, β -myrcene, β -phellandrene, and D-limonene, and oxygenated members, such as 1,8-cineole, isomenthone, dihydrocarvone, and carvone, collectively

Table 3. Major compounds (above 0.5 %) of the essential oil of different Iranian spearmints

Compound (%) *	RT (min)	S 1	S 2	S 3	S 4	S 5	S 6	S 7	S 8
α -pinene	4.2	1.9	1.5	2.1		0.9	1.1	1.5	1
Camphene	5			0.8					
β -pinene	5.9	2.2	1.4	1.7		0.9	0.8	1.2	1.3
β -phellandrene	6.2	1.4	1	1.1		0.6	0.5	0.8	
β -myrcene	7.3	0.9	0.8	1.6		1	1.3	0.7	
D-limonene	8	17.1	8.1	23.1	12.0	28.7	25.9	21.3	34.2
1,8-cineole	8.2	13.6	4.4	7	66.8	7.3		4.6	
<i>Trans</i> - β -ocimene	9								1.7
β -ocimene	9.4								0.6
<i>p</i> -cymene	9.8								1
3-octanol	13.5	0.6	0.9						0.8
1-menthone	15.2		0.7						
Isomenthone	16		1.3						
β -bourbonene	16.7			0.5					
Caryophyllene	18.9	1.2	2	1.3			0.7		1.2
Dihydrocarvone	19.4	1.2	4.3	1.7		0.8	0.5		1.4
Pulegone	20.5		4.2	5.3				4.6	
Dihydrocarveol acetate	21.3					0.7			
Dihydrocarvyl acetate	21.3		1						
D-germacrene	22.1						3		
Isoborneol	22.1					0.6		1.5	2.3
Endo-borneol	22.1			2.8					
Isogermacrene D	22.1		0.9						
α -terpineol	22.2	0.8							
Piperitone oxide	22.3		3.2						
Carvone	23	58.4	29.9	49.6	21.2	56.8	64.7	62.3	50.8
Dihydrocarveol	23.5					1.1	0.6		2.4
<i>Cis</i> -carvone oxide	25.7	0.7							
<i>p</i> -mentha-1,8-dien-3-one	25.7		1.3						
<i>Trans</i> -carveol	25.8			0.5					
Carveol	26.6								1.2
<i>p</i> -menthane-1,2,3-triol	27.6		2.7						
Piperitenone	27.8		4.7	0.9		0.6		0.90	
Carvone oxide (low resolution)	28.9		25.7						
Unidentified	30.3						0.7	0.6	
Total identified (%)		100.0	100.0	100.0	100.0	100.0	99.8	100.0	99.9

* Relative amount in %, dominant components are in bold

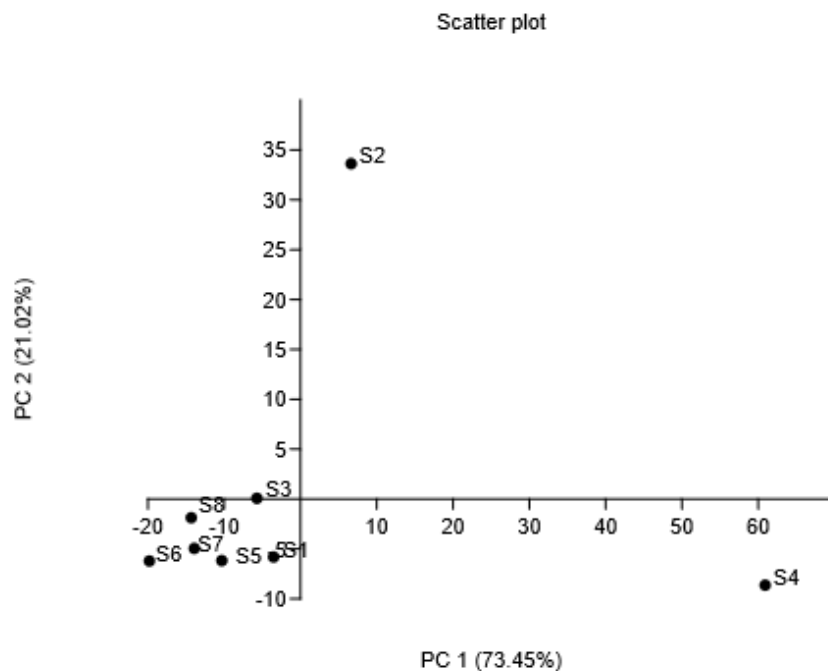


Figure 2. PCA of *Mentha spicata* essential oils collected from different regions of Iran. The scatter plot (PC 1 vs. PC 2) shows the distribution of samples based on their essential oil composition. Samples: S1 – Sonqur (Kermanshah), S2 – Mashhad, S3 – Ilam, S4 – Shaft (Gilan), S5 – Kermanshah, S6 – Sangar (Gilan), S7 – Iranshahr (Sistan and Baluchistan), S8 – Langrood (Gilan). PC 1 (73.45%) and PC 2 (21.02%) together explain 94.47% of the total variance.

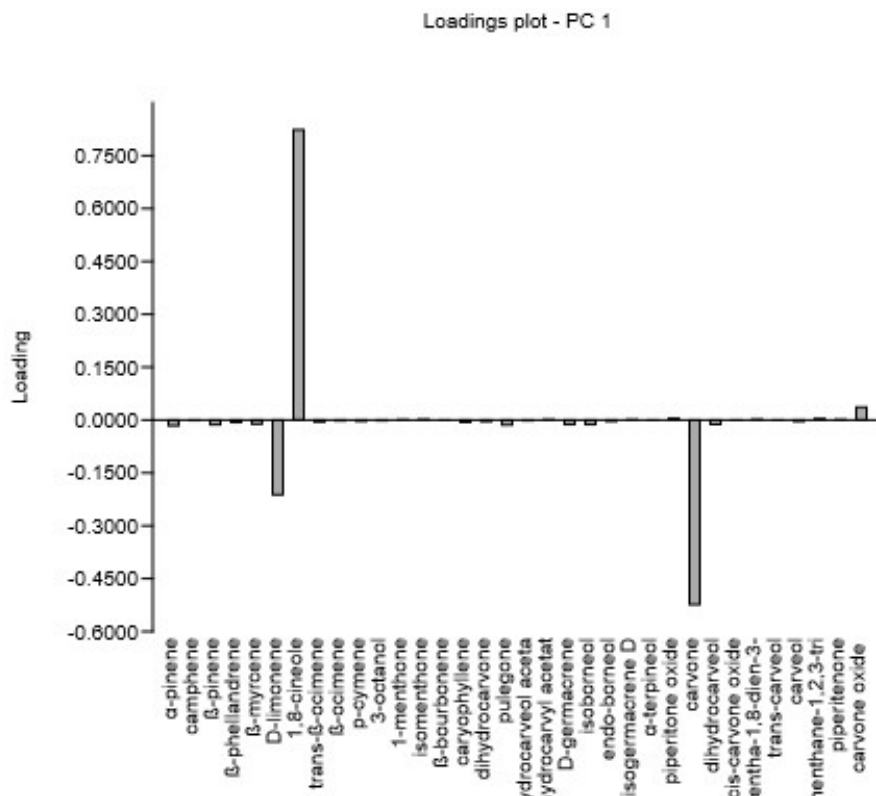


Figure 3. Loading plot of the first principal component (PC 1) obtained from the PCA of *Mentha spicata* essential oils. The plot shows the contribution of individual chemical constituents to the variability explained by PC 1 (73.45%).

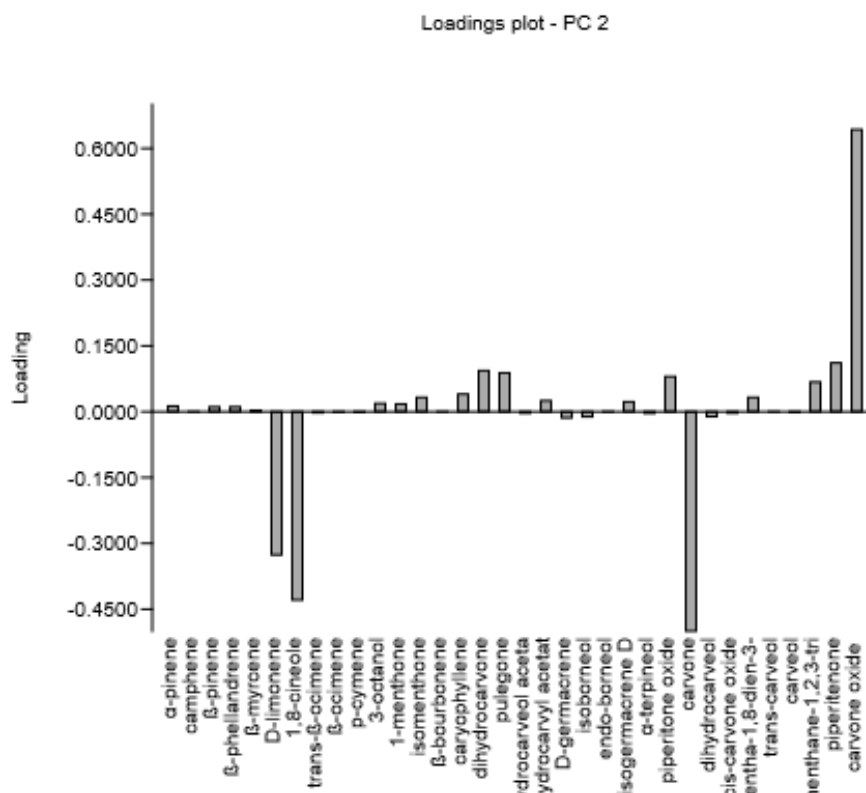


Figure 4. Loading plot of the second principal component (PC 2) obtained from the PCA of *Mentha spicata* essential oils. The plot shows the contribution of individual chemical constituents to the variability explained by PC 2 (21.02%).

accounted for nearly the entire identified fraction in each sample. This dominance was reflected in the consistently high percentages of the major constituents, most notably D-limonene and carvone, whose combined contributions exceeded half of the total monoterpenoid content in all samples. Substantial quantitative variation was observed among samples, with D-limonene ranging from 12.0% in S4 to 34.2% in S8 and carvone ranging from 21.2% in S4 to 64.7% in S6. Sample S4 was distinguished by an exceptionally elevated level of 1,8-cineole (66.8%), a feature that markedly shifted the balance of oxygenated monoterpenoids within this profile. In contrast, sesquiterpenoid hydrocarbons and their oxygenated derivatives were presented only at low levels and showed limited variability across samples. Among these, caryophyllene appeared most consistently, although its contribution never exceeded 2%. Isolated detections of β -bourbonene, D-germacrene, and isogermacrene D were observed, with the latter reaching a maximum of 3% in S3; however, these values remained negligible relative to the overwhelmingly monoterpenoid composition. No meaningful shifts in the distribution of sesquiterpenoid subclasses were detected, underscoring their minor importance within the overall chemical profiles.

The PCA results indicate clear chemical differentiation among *M. spicata* populations from various Iranian regions. PC 1 primarily reflects the inverse relationship between 1,8-cineole and carvone, explaining most of the variance,

with S4 characterized by a higher 1,8-cineole content and the lowest carvone level among the samples. PC 2 highlights variation influenced by D-limonene, 1,8-cineole, carvone, and carvone dioxide. D-limonene, 1,8-cineole, and carvone showed negative loadings on this component, whereas carvone dioxide had a strong positive loading. Sample S2's positive position on PC 2 is mainly due to its uniquely high content of carvone dioxide (25.7%), which was absent in the other samples, demonstrating that the overall variation along this component results from the combined contributions of multiple compounds. These findings suggest the presence of at least two chemotypes among the studied populations along PC 1: a carvone/D-limonene-rich type and a 1,8-cineole-rich type. In addition, variation along PC 2 highlights a third chemical variant, represented by the uniquely high content of carvone dioxide in S2.

Six samples (S1, S3, S5, S6, S7, and S8) displayed a carvone/D-limonene profile, with carvone (~50%–65%) as the principal constituent and D-limonene (~17%–34%) as a major secondary compound. This profile is characteristic of spearmint-type oils and agrees with numerous Iranian *Mentha spicata* studies in which carvone dominates and D-limonene appears as a significant companion constituent (Gandomi Hosnaroodi and Ghavam, 2025; Mahmodi Sorestani and Akbarzadeh, 2015; Shahbazi, 2015). However, the D-limonene fractions in our oils (up to 34%) exceed typical Iranian values (~5%–15%) and are closer to the higher

ranges reported in other regions, such as India and North Africa, where D-limonene may reach 20%–27% (Alsaraf et al., 2021; Mahboub et al., 2025). Such variation is consistent with the known influence of harvest stage, drying, and distillation parameters on volatile monoterpenes like D-limonene, which can shift markedly depending on processing conditions. For example, in Iran, *Mentha spicata* D-limonene content reached ~32.6% in September, compared to much lower values in earlier months (Mahmodi Soarestani and Akbarzadeh, 2015). Also, differences in postharvest drying or hydrodistillation protocols have been shown to substantially alter D-limonene proportions (Moradi-Sadr et al., 2023).

Sample 2 (S2) displayed a mixed composition, distinct from the typical carvone/D-limonene (spearmint-type) oils that dominated most of our samples. Although the carvone content (29.9%) did not reach the levels commonly observed in *Mentha spicata* chemotypes (50%–70%), the sample contained a substantial amount of carvone oxide (25.7%), a close oxidation product of carvone and a known marker associated with spearmint-type oils. D-limonene (8.1%) was likewise lower than the ~15%–30% expected in typical spearmint oils. In addition, S2 contained notable quantities of piperitenone (4.7%) and piperitenone oxide (5.5%), along with minor amounts of pulegone and piperitone, further reflecting a mixed, oxygenated monoterpene profile of a specific genotype for Mashhad region.

Sample 2 exhibited a compositional pattern closely resembling that of an *M. spicata* essential oil from the Ghardaïa region of Algeria, as reported by Laggoune et al. (2016). The Algerian oil was rich in cis-carvone oxide (44.06%), 1,8-cineole (15.32%), cis-dihydrocarvone (8.85%), and limonene (5.80%). Although carvone was absent, both oils are characterized by a pronounced dominance of carvone-oxide–derived constituents. (Laggoune et al., 2016).

Sample 4 showed a 1,8-cineole-dominant composition, with 1,8-cineole (66.8%) as the major compound, accompanied by carvone (21.2%) and D-limonene (12.1%). This profile sets it apart from the carvone/D-limonene (spearmint-type) oils observed in most of the other samples. Although a high content of 1,8-cineole in spearmint is uncommon, a similar spearmint chemotype has been reported by Cook et al. among *Mentha spicata* populations originating from Zakynthos, Greece (Cook et al., 2007). Likewise, a comparably

high proportion of 1,8-cineole was identified in *M. spicata* subsp. *spicata* collected from eastern Turkey (Şarer et al., 2011).

No sample exhibited a peppermint-type menthol/menthone signature, in line with Iranian peppermint data where menthol and menthone dominate (Motiee and Abdoli, 2021; Taherpour et al., 2017).

Overall, our findings highlight three main chemotype patterns among Iranian *Mentha* samples: carvone/D-limonene, mixed carvone/carvone oxide, and 1,8-cineole-rich. These results reinforce the importance of genetic, environmental, and processing factors in shaping essential oil composition. They also provide a comparative framework for linking Iranian *Mentha* chemotypes with their potential pharmaceutical, culinary, and industrial applications.

CONCLUSION

This study demonstrated significant chemical variability among essential oils extracted from eight *Mentha spicata* populations collected across Iran. GC-MS and PCA analyses revealed clear differentiation among samples, indicating the presence of at least two distinct chemotypes: a carvone-limonene (spearmint-type) and a 1,8-cineole-rich type, with one intermediate mixed carvone/carvone oxide profile. The predominance of oxygenated monoterpenes, particularly carvone, D-limonene, and 1,8-cineole, highlights the biochemical diversity of Iranian *M. spicata*. Variations in composition appear to reflect both genetic background and environmental or postharvest factors influencing essential oil biosynthesis. These findings contribute to a better understanding of *M. spicata* chemotypic diversity in Iran and may support the targeted selection of high-value chemotypes for pharmaceutical, flavor, and aromatic applications.

CONFLICTS OF INTEREST

The authors have declared no conflicts of interest.

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