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Original Article

A comparative study of the chemical composition and antioxidant capacity of the essential oils from three species of *Mentha* cultivated in Morocco

[Estudio comparativo de la composición química y la actividad antioxidante de los aceites esenciales de tres especies de *Mentha* cultivadas en Marruecos]

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Abstract

Context: *Mentha* species are well known for their health benefits. Products extracted from aromatic plants of this genus (*Mentha*) are increasingly being studied for their active constituents in order to replace synthetic products that are harmful to health.

Aims: To determine the chemical composition, physicochemical parameters, and antioxidant properties of essential oils of *M. spicata*, *M. piperita*, and *M. pulegium*. These plants were collected from three different geographical areas in the Rabat-Sale-Kenitra region of Morocco, with an additional focus on analyzing the fluctuation of their chemical composition based on their locations.

Methods: Essential oils obtained through hydrodistillation of the fresh aerial parts of the plants were analyzed via gas chromatography and gas chromatography-mass spectrometry. The antioxidant capacity was measured by several chemical tests: DPPH, ABTS, and FRAP.

Results: Multiple major components were identified, showcasing variations in composition between species as well as between plants of the same species. *M. spicata* was characterized by carvone, piperitone, 1,3,8-p-menthatriene; while *M. piperita* features linalool, D-carvone, 1,3,8-p-menthatriene; and *M. pulegium* had a single major component which is pulegone. To the best of our knowledge, it is assumed that a new set of chemotypes may be defined based on the geographical regions studied. The examined essential oils demonstrated notable antioxidant efficacy.

Conclusions: These findings suggest the potential use of extracts from these plants as an alternative to synthetic chemical products. Therefore, they could find applications in complementary medicine as well as in the pharmaceutical and food industries.

Keywords: antioxidant capacity; chemical composition; essential oils; *Mentha piperita*; *Mentha pulegium*; *Mentha spicata*.

Resumen

Contexto: Las especies de *Mentha* son bien conocidas por sus beneficios para la salud. Los productos extraídos de las plantas aromáticas de este género (*Mentha*) son cada vez más estudiados por sus constituyentes activos con el fin de sustituir los productos sintéticos perjudiciales para la salud.

Objetivos: Determinar la composición química, los parámetros fisicoquímicos y las propiedades antioxidantes de los aceites esenciales de *M. spicata*, *M. piperita* y *M. pulegium*. Estas plantas se recolectaron en tres zonas geográficas diferentes de la región marroquí de Rabat-Sale-Kenitra, con el objetivo adicional de analizar la fluctuación de su composición química en función de su ubicación.

Métodos: Los aceites esenciales obtenidos por hidrodestilación de las partes aéreas frescas de las plantas se analizaron mediante cromatografía de gases y cromatografía de gases-espectrometría de masas. La capacidad antioxidante se midió mediante varias pruebas químicas: DPPH, ABTS y FRAP.

Resultados: Se identificaron múltiples componentes principales, mostrando variaciones en la composición entre especies, así como entre plantas de la misma especie. *M. spicata* se caracterizaba por carvona, piperitona, 1,3,8-p-menthatrieno; mientras que *M. piperita* presenta linalol, D-carvona, 1,3,8-p-menthatrieno; y *M. pulegium* tenía un único componente principal que es la pulegona. Hasta donde sabemos, se supone que puede definirse un nuevo conjunto de quimiotipos en función de las regiones geográficas estudiadas. Los aceites esenciales examinados demostraron una notable eficacia antioxidante.

Conclusiones: Estos hallazgos sugieren el uso potencial de los extractos de estas plantas como alternativa a los productos químicos sintéticos. Por lo tanto, podrían encontrar aplicaciones en medicina complementaria, así como en las industrias farmacéutica y alimentaria.

Palabras Clave: aceites esenciales; capacidad antioxidante; composición química; *Mentha piperita*; *Mentha pulegium*; *Mentha spicata*.

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Abbreviations: ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); A.F.N.O.R: the standards organization of France; BHA: butylated hydroxyanisole; BHT: butylated hydroxytoluene); DMSO: dimethylsulfoxide; DPPH: 2,2-diphenyl 1-picrylhydrazyl; EOs: essential oils; FID: flame ionization detector; FRAP: ferric reducing antioxidant power assay; IC_{50}: Inhibitory concentration of 50%; GC: gas chromatography; GC-MS: gas chromatography-mass spectrometry; RI: retention index.

INTRODUCTION

Mentha is a plant that is indigenous to the Mediterranean region. It is cultivated worldwide for its diverse applications. It belongs to the *Lamiaceae* family and has a variety of species, which are widely distributed and cultivated in temperate tropical regions (Fazili et al., 2020). It is extensively used in Mediterranean regions as a food flavoring and for therapeutic purposes to treat common colds, stomachaches, hemorrhoids, and upper respiratory tract infections (Tawaha et al., 2007). Nowadays, it is considered to be an industrial product, with its essential oils being used in the pharmaceutical, food, beverage, confectionery, toothpaste, mouth freshener, and cosmetics industries (Alsaraf et al., 2021).

Mentha spicata (spearmint), *Mentha pulegium* (pennyroyal mint), and *Mentha piperita* (peppermint) are the most important aromatic medicinal plants within this genus. The extracts derived from these plants have been extensively studied for their antibacterial, antifungal, anti-inflammatory, and antioxidant properties (Arrahmouni et al., 2023; Boukhobza, 2020; Chraibi et al., 2016).

M. spicata, *M. pulegium* and *M. piperita* essential oils (EOs) are diverse mixtures of phenolics, tannins, terpenes, terpenoids, quinones, coumarins, flavonoids, alkaloids and sterols (Ait-Sidi-Brahimet al., 2019; El-Gharbaouiet al., 2017). Studies have shown that *M. pulegium* has highly efficacious antifungal activity against *Candida albicans*, inhibiting biofilm formation, growth, transition, and the expression of virulence-related genes (Benzaid et al., 2019). Other studies highlighted the bactericidal power of *M. spicata* and *M. piperita*, and the possibility of using their EOs as natural antibiotics (Ben Lagha et al., 2020; Rasooli et al., 2009). The antioxidant properties of EOs and their constituents have been widely studied by several researchers, who have revealed that these EOs can be used as natural antioxidants to replace butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) and other synthetic conservants that are known to pose risks to human health (Kahl and Kappus, 1993; Ouedrhiri et al., 2021; Singh et al., 2015).

However, the composition of the extracted products (EOs) can be altered by several factors influencing the plant, such as climate, soil composition, plant organ, age, and stage of the vegetation cycle, as well as by different species and chemotypes, and the EO extraction technique (Elbouny et al., 2022; Palá-Paúl et al., 2001). These environmental factors and plant characteristics are important criteria for studying the activity of essential oils.

The purpose was to evaluate the chemical composition by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) of *M. spicata*, *M. piperita and M. pulegium,* and analyze their variabilities according to three different geographical areas in the Rabat-Sale-Kenitra region. In addition, the aim was to evaluate the physicochemical parameters and antioxidant capacity of these EOs.

MATERIAL AND METHODS

Chemicals and reagents

The chemicals and reagents used in this investigation included 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis acid (ABTS), Ferric reducing antioxidant power assay (FRAP), dimethyl sulfoxide (DMSO), quercetin, catechin, and ascorbic acid, all obtained from Sigma-Aldrich (St. Louis, MO, USA). Additionally, potassium ferricyanide ($K_3Fe(CN)_6$), phosphate buffer, trichloroacetic acid, potassium persulfate, anhydrous sodium sulfate, and methanol were acquired from COGELAB (Morocco).

Plant material

The three mint varieties: *M. spicata*, *M. piperita* and *M. pulegium* were collected from three different areas in the Rabat-Sale-Kenitra region. Samples from the Akkari area (34°00'29"N 6°51'19"W) were collected during July 2022, while samples from Sidi Ayach (34°19'59"N 6°27'26"W) and Ain Atiq (33°53'38"N 6°58'00"W) areas were collected during October-November 2022. Botanical identification of the plant material was conducted at the Department of Botany, Faculty of Sciences and Techniques of Errachidia (FSTE). The extraction of EOs was carried out using the leaves of *M. spicata* and *M. piperita*, as for *M. pulegium*, the aerial floral parts were used.

Extraction

A total of 500 g of each botanical material underwent hydrodistillation in 1 L of distilled water for a duration of 3 h utilizing a Clevenger-type apparatus as per the guidelines outlined in the European Pharmacopoeia (Council of Europe, 2007). Subsequently, the EOs obtained were dried using anhydrous sodium sulfate and preserved in light-proof bottles at a temperature of 4 ± 1°C until further use.

Gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) analysis

GC and GC-MS were used to analyze the essential oil that was obtained.

GC

GC analyses were performed using a Shimadzu GC-2010 Plus gas chromatograph equipped with a flame ionization detector (FID) and a DB-5 capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm SGE Ltd). The oven temperature was programmed to increase from 60°C to 200°C, 3°C.min^{-1}, followed by an isothermal hold for 5 minutes.

The injector and detector temperatures were set at 280°C and 300°C, respectively. The carrier gas, nitrogen, was adjusted to achieve a linear velocity of 30 cm/s. Sample injection was performed using a split sampling technique with a ratio of 1:50. The injection volume was 0.2 µL of a pentane-volatile solution (1:1).

GC-MS

The GC-MS unit utilized a Shimadzu GC-2010 gas chromatograph, equipped with a BP-5 capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm; SGE, Ltd.), and interfaced with a Shimadzu QP2010 Plus mass spectrometer (software version 2.50 SU1). The oven temperature was programmed following the described parameters for GC analysis. The transfer line temperature was set at 300°C, ion source temperature at 200°C, and the carrier gas (helium) adjusted to a linear velocity of 36.5 cm/s. The split ratio was 1:40, ionization energy was at 70 eV, the scan range was from 40 to 400 m/z, and the scan time was 1 s.

Component identification was achieved by comparing their retention indices relative to C9-C20n alkanes on the DB-5 column (Adams, 2007), further confirmed through a comparison of recorded mass spectra with those available in a computer library (Shimadzu corporation library and NIST05 database/ChemStation data system), as well as from a home-made library. The latter was constructed based on analyses of reference oils, laboratory-synthesized components, commercially available standards, and other literature data (Elouaddari et al., 2019; Laseve, 1996).

Antioxidant capacity

DPPH test

The test is based on DPPH free radical scavenging. The EO samples were solubilized in DMSO, and successive dilutions were prepared from the initial extract. Subsequently, 50 µL of each EO dilution was mixed with 2 mL of methanolic DPPH solution (60 µM). The mixture was vortexed and incubated at room temperature for 20 min. The absorbance of the samples was measured at 517 nm against a control. A control was also prepared using DMSO and DPPH solution. Quercetin served as a standard antioxidant, following the same conditions as the EOs (Sahin et al., 2004). The test was conducted in triplicate, and the percentage of inhibition was calculated using the following equation [1].

$$\text{Inhibition (\%)} = \frac{A_{control} - A_{sample}}{A_{control})} * 100 \quad [1]$$

Where: $A_{control}$: Absorbance of the mixture containing the DPPH solution with the DMSO used to solubilise the samples. A_{sample}: Absorbance of the mixture containing the DPPH solution and the sample.

The results were expressed as an inhibitory concentration of 50% DPPH (IC_{50}) using the regression equation obtained by plotting the concentration as a function of the percentage of inhibition.

FRAP test

This assay is based on the reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}). The FRAP reagent and concentrations of EOs were prepared. To perform the assay, 0.2 mL of each sample, 2.5 mL of phosphate buffer (0.2 M and pH = 6.6), and 2.5 mL of 1% (w/v) potassium ferricyanide complex ($K_3Fe(CN)_6$) were mixed and incubated at 50°C for 20 min. After adding 2.5 mL trichloroacetic acid at 10%, absorbance was measured at 700 nm (Oyaizu, 1986). The test was carried out in triplicate. The results were obtained by plotting the concentration as a function of the absorbance of the sample. The effective concentration of 50% Fe^{3+} to Fe^{2+} (EC_{50}) was obtained by exploiting the regression equation obtained. Consequently, the results obtained were compared with those of the catechin, which were taken as reference standards.

ABTS test

The antioxidant capacity of EOs was also assessed by ABTS. The ABTS reagent was prepared 16 h before the manipulation by mixing 10 mL of a 7 mM methanolic solution of ABTS and 10 mL of a 70 mM methanolic solution of potassium persulfate. This solution was then diluted with methanol until an absorbance between 0.700 and 0.734 nm was obtained. Successive concentrations of extracts were prepared in DMSO and mixed with the reagent. After incubation for 1 min at room temperature, absorbance was registered at 734 nm (Pukalskas et al., 2002). The test was performed in triplicate, and the percentage inhibition was calculated using the equation [1], as the DPPH test. The results were expressed as an inhibitory concentration of 50% ABTS (IC_{50}) using the regression equation obtained by plotting the concentration as a function of the percentage of inhibition. In addition,

the results obtained were compared with those of ascorbic acid, which was taken as a reference standard.

Analytical study of EO

Organoleptic properties of EOs

The various organoleptic characteristics (flavor, color, and odor) of different varieties of EOs were evaluated.

Measurement of physicochemical parameters

The analyses were carried out in accordance with AFNOR standards (AFNOR, 1998) as physical characteristics: density, refractive index, rotatory power, and chemical characteristics: acid value, ester value, saponification value, and peroxide value. The test was evaluated in triplicate, and the results were presented as mean ± standard deviation.

Statistical analysis

The experiments were performed in triplicate, and the results were presented as the mean ± standard deviation (SD). Statistical analysis and mean comparisons were conducted using GraphPad Prism v8 software, utilizing one-way analysis of variance (ANOVA) followed by the Tukey test. Statistical significance was considered for p-values less than 0.05.

RESULTS

Organoleptic properties of EOs

Odor, color, and flavor were evaluated. The results are shown in Table 1.

Variation in physicochemical parameters

The results of the physicochemical parameters are presented in Table 2.

Table 1. Organoleptic characteristics of the EOs studied.

Sample	Origin	Odor	Color	Flavor
M. spicata	Ak	+	Yellow	Minty +
	SA	++	Yellow	Minty +
	AA	+	Yellow	Minty +
M. piperita	Ak	++	Yellow	Minty ++
	SA	++	Yellow	Minty++
	AA	++	Translucent	Minty++
M. pulegium	Ak	+++	Green-yellow	Minty +++
	SA	+++	Green-yellow	Minty +++
	AA	+++	Green-yellow	Minty +++

Ak: Akkari; SA: Sidi Ayach; AA: AinAtiq.

Table 2. Yield and physicochemical parameters of *Mentha species* EOs.

Origin	Y%	D (g/mL)	RI	RP	AV (mg KOH/g)	SV (mg KOH/g)	EV (mg KOH/g)	PV (mg Eq 0_2/g)
M. spicata								
Akkari	1.10	0.892 ± 0.02^a	1.478 ± 0.04^a	-59 ± 0.04^a	1.30 ± 0.19^a	112.20 ± 0.45^a	110.90 ± 0.89^a	0.739 ± 0.02^a
Sidi Ayach	0.63	0.865 ± 0.01^a	1.478 ± 0.10^a	-68 ± 0.12^b	0.09 ± 0.02^b	84.15 ± 0.32^b	84.06 ± 0.68^b	0.459 ± 0.06^b
Ain Atiq	0.63	0.878 ± 0.03^a	1.462 ± 0.02^a	-44 ± 0.22^c	0.09 ± 0.01^b	56.10 ± 0.07^c	56.01 ± 0.33^c	0.520 ± 0.03^c
M. piperita								
Akkari	0.50	0.929 ± 0.07^a	1.460 ± 0.05^a	-17 ± 0.07^a	1.20 ± 0.03^a	56.10 ± 0.09^a	54.90 ± 0.10^a	1.33 ± 0.10^a
Sidi Ayach	0.15	0.931 ± 0.03^a	1.469 ± 0.01^a	-26 ± 0.05^b	0.65 ± 0.03^b	56.10 ± 0.11^a	55.45 ± 0.07^b	1.23 ± 0.09^a
Ain Atiq	0.15	0.909 ± 0.01^a	1.461 ± 0.02^a	-21 ± 0.01^c	0.60 ± 0.03^b	78.54 ± 0.10^b	77.94 ± 0.10^c	1.45 ± 0.07^a
M. pulegium								
Akkari	1.75	0.938 ± 0.11^a	1.485 ± 0.07^a	+21 ± 0.54^a	2.36 ± 0.15^a	14.02 ± 0.8^a	11.66 ± 0.35^a	8.02 ± 0.10^a
Sidi Ayach	1.47	0.954 ± 0.10^a	1.486 ± 0.04^a	+21 ± 0.23^a	2.00 ± 0.13^a	42.07 ± 0.34^b	40.07 ± 0.67^b	5.44 ± 0.13^b
Ain Atiq	0.80	0.925 ± 0.09^a	1.485 ± 0.02^a	+16 ± 0.29^b	1.12 ± 0.17^b	22.44 ± 0.045^c	20.03 ± 0.57^c	5.00 ± 0.23^b

Y: Yield; D: Density; RI: Refractive index; RP: Rotating power; AV: Acid value; SV: Saponification value; EV: Ester value; PV: Peroxide value. Results were expressed as mean ± standard deviation (n = 3). Values in the same column with different superscript letters indicate significant differences ($p<0.05$).

Chemical composition

Akkari area

Table 3 presents the retention indices and percentage composition of the identified compounds in the EOs of *M. spicata*, *M. piperita*, and *M. pulegium*, respectively, collected from the Akkari area.

Regarding *M. spicata*, significant variations in chemical composition were observed among plant populations. Fifteen compounds, constituting 100% of the oil, were identified. The primary constituents included carvone (58.16%), limonene (22.4%), 1,8-cineole (6.84%), and β-myrcene (3.51%). The chromatogram in Fig. 1 illustrates the GC-MS analysis (Fig. 1A).

A total of 25 constituents representing 98.59% of the total EOs of *M. piperita* were identified. The main components were linalool (59.34%), linalool acetate (12.59%), α-terpineol (6.93%), 1,8-cineole (3.57%), 1-tetradecene (3.49%), and geraniol (2.48%) (Table 3, Fig. 1B).

Regarding *M. pulegium*, 28 constituents representing 99.75% of the total EOs were identified. The main components were the pulegone, which represents (49.88%), isomenthone (25.26%), and cis-β-ocimene (4.28%) (Table 3, Fig. 1C).

Sidi Ayach area

Table 4 lists the retention indices and percentage composition of the compounds identified in the *M. spicata*, *M. piperita,* and *M. pulegium* EOs collected from the Sidi Ayach area.

In regard to *M. spicata*, 21 components representing 100% of the EOs were identified. The major components were piperitone (36.68%), 1,3,8-ρ-menthatriene (25.68%), 2-(1E)-propenyl-phenol (5.27%), 5methyl-hexanoic acid (4.99%), menthan-2-one (3.27%), cis-piperitol (3.09%) (Table 4, Fig. 2A).

The chemical composition of *M. piperita* revealed 19 components representing 100% of the EOs. This peppermint variety was dominated in this case by D-carvone (56.75%), citronellol (11.03%), maltol (10.38%), 5-methyl-hexanoic acid (7.39%), and 2-(1E)-propenyl-phenol (1.73%) (Table 4, Fig. 2B).

About *M. pulegium*, 21 components were identified, representing 99.99% of the EOs. The predominant component was pulegone, accounting for 81.54%, with the other remaining components listed as follows: Mentho thiophene (4.32%), pentyl-benzene (2.57%), 1-tridecene (1.96%) and, menthol (1.04%) (Table 4, Fig. 2C).

Ain Atiq area

Table 5 lists the retention indices and percentage composition of the identified compounds in the *M. spicata*, *M. piperita* and *M. pulegium* EOs collected in the Ain Atiq area.

The chemical analysis of the EOs of *M. spicata* revealed 17 components representing 100% of the EO. The major components were piperitone (77.84%), 5methyl-hexanoic acid (5.61%), benzene acetaldehyde (2.69%), citronellol (2.49%) and maltol (2.29%)(Table 5, Fig. 3A).

Regarding *M. piperita*, 24 components were identified; they amount to 100% of the EOs. The most important constituents are as follows: 1,3,8-ρ-menthatriene (56.34%), 2-(1E)-propenyl-phenol (13.96%), cis-piperitol (8.19%), (E)-α-damascone (3.95%), trans-ρ-menth-6-en-2,8-diol (1.95%) and pulegone (1.28%) (Table 5, Fig. 3B).

The results drawn from *M. pulegium* showed 20 constituents representing 100% of the EOs. A significant proportion of pulegone (75.34%) was observed. Other components were identified with lower percentages, namely terpineol (7.06%), mentho thiophene (4.94%), 1-tridecene (1.81%), and menthol (1.55%) (Table 5, Fig. 3C).

Antioxidant capacity

DPPH

The results of the DPPH antioxidant capacity showed better performance of *M. spicata* from the Ain Atiq area, followed by *M. piperita* from the Sidi Ayach area. However, this activity was significantly lower ($p<0.05$) than the commercial standard used, quercetin (0.0054 ± 0.0001 mg/mL). On the other hand, some EOs were inactive under this technique (Table 6).

ABTS

In terms of *M. spicata* varieties, the Sidi Ayach area showed the highest antioxidant capacity. However, as to *M. piperita*, the Ain Atiq area demonstrated significantly high ($p<0.05$) activity, while the Sidi Ayach and Akkari areas were inactive. Nevertheless, the best antioxidant capacity was recorded in the Sidi Ayach area variety of *M. pulegium*, followed by Ain Atiq and Akkari. All varieties had significantly low ($p<0.05$) antioxidant capacity compared with the ascorbic acid standard used (0.0025 ± 0.0002 mg/mL) (Table 6).

FRAP

According to Table 6, all the samples studied exhibited activity. Concerning *M. spicata*, two varieties,

from the Sidi Ayach and Akkari areas, demonstrated significantly high activity ($p<0.05$).

In the case of *M. piperita*, the most notable activity was observed in the Sidi Ayach EOs, showing a significant difference ($p<0.05$) compared to the others. For *M. pulegium*, the Akkari variety displayed significantly the highest activity ($p<0.05$), followed by Sidi Ayach and Ain Atiq. On the other hand, catechin showed significantly higher ($p<0.05$) antioxidant capacity than the other samples (0.013 ± 0.0025 mg/mL).

Table 3. Chemical composition of the EOs of the *M. spicata*, *M. piperita*, and *M. pulegium* collected from the Akkari area.

Component	RI[a]	RI[b]	Percentage
M. spicata			
α-Pinene	942	932	1.32
Camphene	956	946	0.16
Sabinene	982	969	0.91
β-Pinene	986	974	1.76
β-Myrcene	1001	988	3.51
α-Phellandrene	1004	1002	0.25
Limonene	1035	1026	22.40
Eucalyptol	1037	1039	6.84
Borneol	1169	1165	0.35
trans-Dihydrocarvone	1202	1200	2.01
Citronellol	1224	1223	0.56
Pulegone	1243	1233	0.29
Carvone	1251	1239	58.16
β-Caryophyllen	1422	1417	0.67
D-Germacrene	1484	1484	0.81
M. piperita			
β-Pinene	981	974	0.17
β-Myrcene	1001	988	2.60
Limonene	1034	1024	0.52
Eucalyptol	1036	1036	3.57
β-trans-Ocimene	1043	1044	0.73
γ-Terpinene	1053	1054	0.40
Terpinolen	1078	1086	0.14
Linalool	1109	1095	59.34
trans-Thujone	1112	1112	0.22
1-Octen-3-yl-acetate	1119	1117	0.17
trans-Pinocarveol	1131	1135	0.20
Isomenthone	1158	1153	0.16
α-Terpineol	1196	1186	6.93
Pulegone	1234	1233	0.97
p-Anisaldehyde	1243	1247	0.44
Geraniol	1261	1249	2.48
Linalool acetate	1263	1254	12.59

Table 3. Chemical composition of the EOs of the *M. spicata, M. piperita*, and *M. pulegium* collected from the Akkari area (continued...)

Component	RI[a]	RI[b]	Percentage
Geranial	1276	1264	0.13
Eugenol	1356	1356	0.42
α- Copaene	1373	1374	1.71
1-Tetradecene	1392	1388	3.49
(Z)-Caryophyllene	1397	1409	0.13
α-trans-Bergamotene	1422	1432	0.26
Hedycaryol	1554	1546	0.53
1-Hexadecene	1593	1588	0.29
M. pulegium			
α-Pinene	941	932	0.90
β-Pinene	981	974	1.64
β-Myrcene	1001	974	0.70
alpha-Phellandrene	1004	988	0.24
cis-beta-Ocimene	1034	1002	4.28
Eucalyptol	1036	1032	1.18
trans-beta-Ocimene	1043	1039	0.53
Linalool	1107	1044	0.47
Isomenthone	1158	1095	25.26
Borneol	1168	1153	3.04
alpha-Terpineol	1180	1165	2.87
Pulegone	1245	1186	49.88
Linaloolacetate	1259	1232	1.30
Geranial	1267	1254	0.13
Bornyl acetate	1286	1264	0.12
2-Undecanone	1293	1284	0.45
Terpinylacetate	1343	1293	1.39
alpha Copaene	1370	1346	1.97
1-Tetradecene	1397	1374	0.12
beta-Caryophyllene	1422	1388	1.03
alpha-Caryophyllene	1456	1417	0.18
Humulene alpha	1465	1444	0.32
Germacrene D	1484	1452	1.26
delta-Cadinene	1527	1484	0.41
Spathulenol	1579	1522	0.14
Caryophyllene oxide	1584	1577	0.21
beta-Himachalenoxide	1618	1582	0.14

[a]Experimental Linear retention index. [b]Relative Linear retention index to C_9–C_{22}*n*-alkanes on the DB-5 column taken from (Adams, 2007) for DB-5 capillary column in literature.

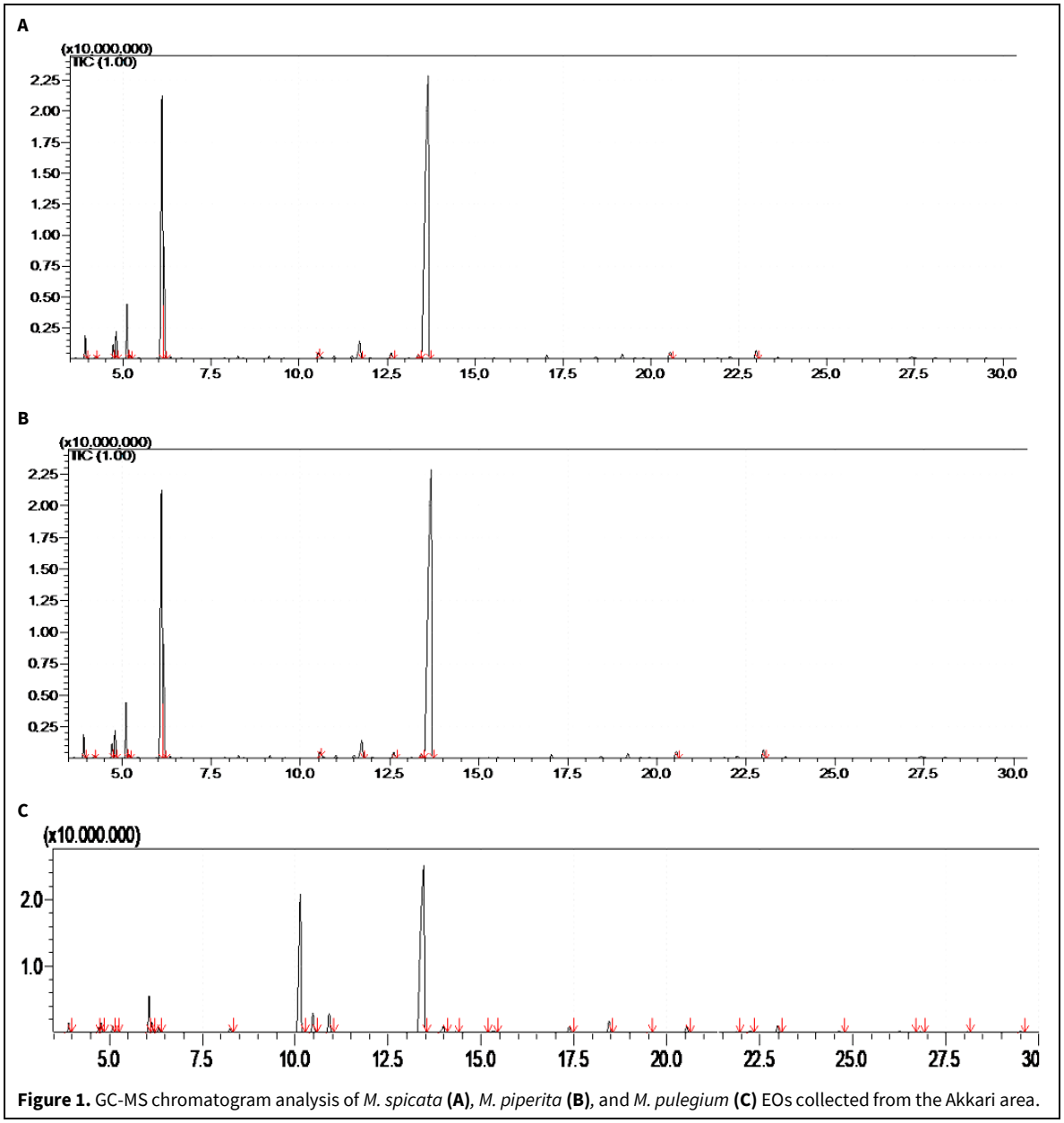

Figure 1. GC-MS chromatogram analysis of *M. spicata* **(A)**, *M. piperita* **(B)**, and *M. pulegium* **(C)** EOs collected from the Akkari area.

Table 4. Chemical composition of the EOs of *M. spicata, M. piperita and M. pulegium* collected from the Sidi Ayach area.

Component	RI[a]	RI[b]	Percentage
M. spicata			
α-Pinene	940	932	0.35
cis-Pinane	985	984	0.89
meta-Mentha-1(7),8-diene	1000	1000	2.55
5methyl-Hexanoic acid	1034	1033	4.99
Benzene acetaldehyde	1036	1036	3.60
γ-Hexalactone	1042	1042	0.81
Isopoentyl butanoate	1053	1052	0.38
1,3,8-ρ-Menthatriene	1108	1108	25.68

Table 4. Chemical composition of the EOs of *M. spicata, M. piperita and M. pulegium* collected from the Sidi Ayach area (continued...)

Component	RI[a]	RI[b]	Percentage
1-Terpineol	1130	1130	0.90
cis-Piperitol	1195	1195	3.09
Menthan-2-one	1200	1199	3.27
cis-4-Caranone	1201	1201	1.90
Citronellol	1224	1223	2.74
Piperitone	1250	1249	36.86
(2E)- Decenal	1261	1260	1.42
2-(1E)-propenyl-Phenol	1263	1264	5.27
Linalool propanoate	1334	1334	1.20
Trans-Menth-6-en-2,8-diol	1372	1371	0.64
(E)-α-Damascone	1391	1392	1.33
ρ-Menth-1-en-9-ol acetate	1421	1421	1.35
α-Amorphene	1483	1483	0.78
M. piperita			
cis-Pinane	985	982	0.73
meta -Mentha-1(7),8-diene	1001	1000	1.48
Phellandrene	1004	1002	0.48
5-methyl-Hexanoic acid	1034	1033	7.39
Benzene acetaldehyde	1036	1036	1.52
Maltol	1107	1106	10.38
1-Terpineol	1131	1130	0.46
cis-Piperitol	1195	1195	1.18
γ-Terpineol	1200	1199	1,19
trans-Dihydro carvone	1201	1201	1.06
Citronellol	1224	1223	11.03
D-Carvone	1250	1250	56.75
(2E)-Decenal	1261	1261	0.46
2-(1E)-propenyl-Phenol	1263	1264	1.73
trans-Piperitol acetate	1344	1343	0.46
α-Isocomene	1388	1388	0.76
(E)-α-Damascone	1392	1392	0.48
ρ-Menth-1-en-9-ol acetate	1412	1421	1.21
α-Amorphene	1483	1483	1.25
M. pulegium			
α-Pinene	940	932	0.11
Phellandrene	1003	1002	1.25
5-methyl-Hexanoic acid	1033	1033	0.74
Bergamal	1051	1051	0.22
Maltol	1106	1106	0.28

Table 4. Chemical composition of the EOs of *M. spicata, M. piperita and M. pulegium* collected from the Sidi Ayach area (continued...)

Component	RI[a]	RI[b]	Percentage
pentyl-Benzene	1152	1152	2.57
Terpineol	1156	1156	0.70
Menthol	1167	1167	1.04
ρ-methyl-Acetophenone	1179	1179	1.75
trans-Pulegol	1213	1213	0.16
Thymol, methyl ether	1232	1232	0.23
Pulegone	1245	1245	81.54
2-(1E)-propenyl-Phenol	1266	1264	0.78
6-Undecanol	1285	1285	0.46
1-Tridecene	1291	1291	1.96
Undecanal	1307	1305	0.44
Mentho thiophene	1342	1342	4.32
γ-Elemene	1436	1436	0.41
Maltol propionate	1455	1456	0.26
cis Cadina-1(6),4-diene	1462	1461	0.21
ar-Tumerol	1583	1582	0.17
β-Atlantol	1609	1608	0.39

[a]Experimental Linear retention index. [b]Relative Linear retention index to C_9–C_{22}*n*-alkanes on the DB-5 column taken from (Adams, 2007) for DB-5 capillary column in literature.

DISCUSSION

Physicochemical properties of EOs

According to Tables 1 and 2, hydrodistillation of the aerial part of Akkari *M. spicata* produced yellow EOs with a minty odor. A higher yield was recorded for the Akkari variety (1.10%) than for the other varieties (0.63%). El Hassani et al. (2009) obtained a yield of 1.20%, while Mahboubi (2021) reported that the yield varies between 2.41-2.74%. In light of this, yields are subject to variation due to several factors (Hussain et aI., 2010). The relative density results show a value fluctuating around 0.80 g/mL, which is in line with previous research (Sulieman et al., 2011; Weecharangsan et al., 2014). Concerning the refractive index, the values were almost identical, with the highest value of 1.478 for the Akkari and Sidi Ayach varieties, compared with 1.462 for the Ain Atiq area. The three varieties showed differences in their rotatory power and chemical indices. For example, the acid value was 1.30 mg KOH/g for Akkari *M. spicata* and 0.90 mg KOH/g for the other two, which does not correspond to the results found by Sulieman et al. (2011).

Regarding *M. piperita*, the EOs extracted are characterized by a yellow and translucent color with a distinctive minty odor. A difference in yield was noted between the three varieties, with a higher value of 0.50%, similar to that found in Congo (Likibi et al., 2015). The physical and chemical characteristics of *M. piperita* EOs were determined (Table 2). The results of the obtained parameters showed significant differences.

M. pulegium EOs extracted from aerial parts are characterized by an overly intense odor with a greenish-yellow color. *M. pulegium* showed the highest yield compared to *M. spicata* and *M. piperita*, with a value of 1.75%. However, this rate is relatively low compared with those reported in other studies (Ait-Ouazzou et al., 2012; Zekri et al., 2013). Concerning physical and chemical parameters, the results showed that each variety has its own characteristics (Table 2). In Algeria, Hariri et al. (2020) studied the physicochemical parameters of *M. pulegium* EOs. They found the following results: relative density of 0.908 g/mL, refractive index at 20°C 1.486, rotatory power of +2.25, acid value of 9.537 mg KOH/g, ester value of 46.573 mg KOH/g at 20°C, and saponification value of 56.11. Based on these findings, it is clear that the parameters differ from one study to another and may be influenced by several factors such as the origin of the material, edaphic factors, and climate, among others.

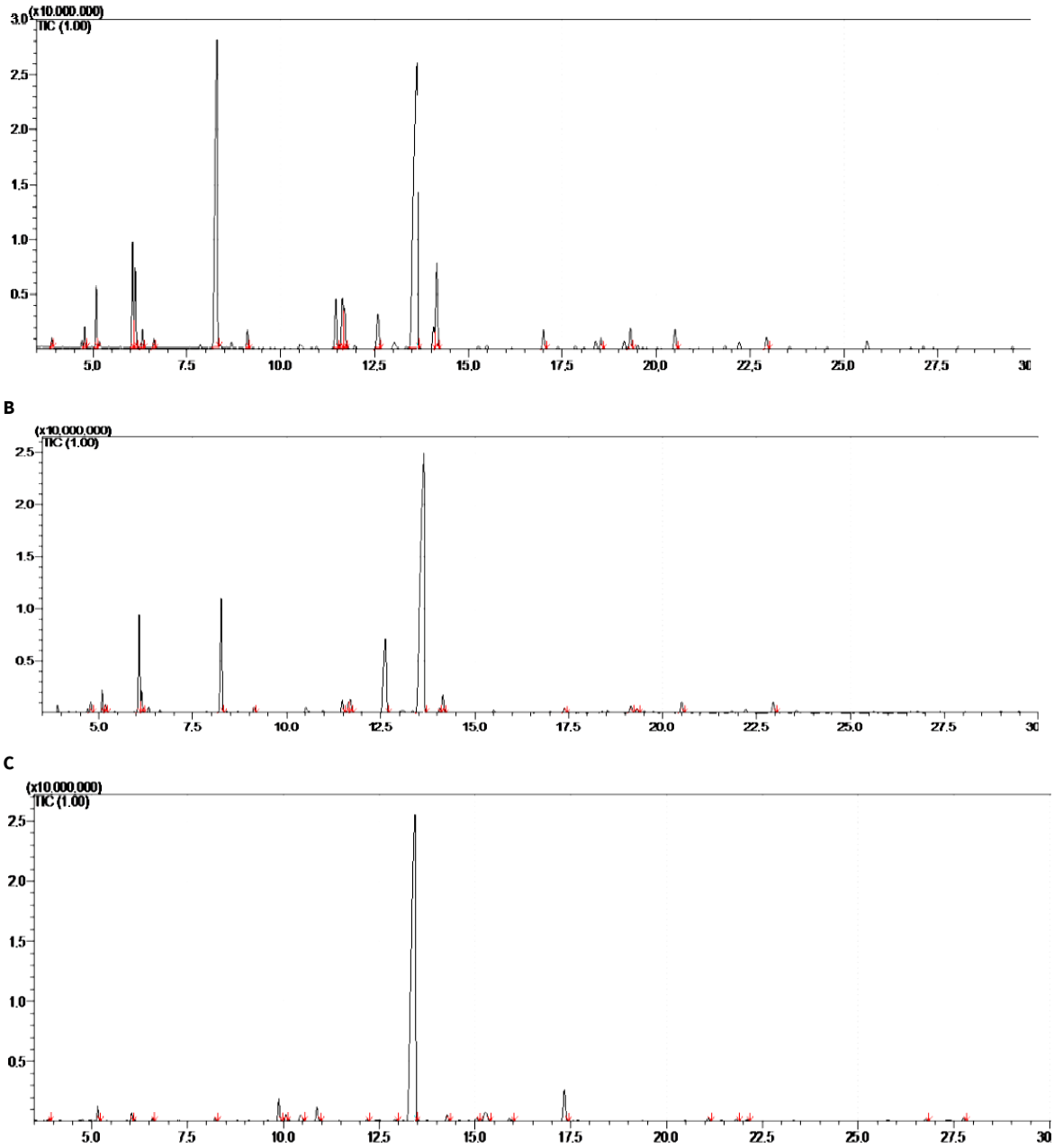

Figure 2. GC-MS chromatogram analysis of *M. spicata* **(A)**, *M. piperita* **(B)**, and *M. pulegium* **(C)** EOs collected from the Sidi Ayach area.

Table 5. Chemical composition of the EOs of *M.spicata*, *M. piperita,* and *M. pulegium* collected from the Ain Atiq area.

Component	RI[a]	RI[b]	Percentage
M. spicata			
α-Pinene	941	932	0.20
trans-meta-Mentha-2,8-diene	981	979	0.19
cis-Pinane	985	984	0.61
meta-Mentha-1(7),8-diene	1000	1000	1.42
ρ-Mentha-1(7),8-diene	1004	1003	0.34

Table 5. Chemical composition of the EOs of *M.spicata*, *M. piperita,* and *M. pulegium* collected from the Ain Atiq area (continued...)

Component	RI[a]	RI[b]	Percentage
5-methyl-Hexanoic acid	1034	1033	5.61
Benzene acetaldehyde	1036	1036	2.69
Maltol	1106	1106	2.29
1-Terpineol	1131	1130	0.48
cis-Piperitol	1195	1195	0.36
Menthan-2-one	1200	1199	1.62
cis-4-Caranone	1201	1201	1.82
Citronellol	1223	1223	2.49
Piperitone	1284	1249	77.84
Linalool propanoate	1334	1334	0.50
ρ-Menth-1-en-9-ol acetate	1421	1421	0.91
α-Amorphene	1483	1483	0.64
M. piperita			
meta-Mentha-1(7),8-diene	1000	1000	1.25
5-methyl-Hexanoic acid	1034	1033	0.24
Benzene acetaldehyde	1036	1036	2.20
γ-Hexalactone	1043	1042	0.88
Isopentyl butanoate	1053	1052	0.56
Linalool	1095	1095	0.29
1,3,8-ρ-Menthatriene	1108	1108	56.34
(2E)- Heptenyl acetate	1111	1111	0.16
3-Methylbutanote,3-methyl-butenyl	1115	1112	0.16
1-Terpineol	1131	1130	0.24
cis-Piperitol	1195	1195	8.19
Pulegone	1234	1233	1.28
ρ-Anisaldehyde	1247	1247	0.55
(2E)- Decanal	1261	1260	3.88
2-(1E)-propenyl-Phenol	1263	1264	13.96
4-Hydroxybenzaldehyde	1355	1355	0.42
trans-ρ-Menth-6-en-2,8-diol	1372	1371	1.95
(E)-α-Damascone	1392	1392	3.95
(Z)-Trimenal	1396	1397	0.36
n-Tetradecane	1400	1400	0.22
ρ-Menth-1-en-9-ol acetate	1421	1421	0.76
α-Amorphene	1483	1483	0.26
cis-Cadinene ether	1553	1552	1.51
Viridiflorol	1592	1592	0.39

Table 5. Chemical composition of the EOs of *M.spicata*, *M. piperita*, and *M. pulegium* collected from the Ain Atiq area (continued...)

Component	RI[a]	RI[b]	Percentage
M. pulegium			
Phellandrene	1003	1002	0.95
5-methyl-Hexanoic acid	1033	1033	0.44
Bergamal	1051	1051	0.30
pentyl-Benzene	1152	1152	1.10
Terpineol	1156	1156	7.06
Menthol	1167	1167	1.55
ρ-methyl-Acetophenone	1179	1179	1.88
Pulegone	1244	1245	75.34
trans-Piperitone epoxide	1252	1252	0.28
2-(1E)-propenyl-Phenol	1266	1264	0.83
6-Undecanol	1285	1285	0.55
1-Tridecene	1291	1291	1.81
Undecanal	1307	1305	0.37
Menthol thiophene	1342	1342	4.94
(2E)-Undecanal	1358	1357	0.30
Methyl benzyl butyrate	1363	1363	0.56
2,5-dimethoxy-ρ-Cymene	1424	1424	0.31
γ-Elemene	1436	1436	0.58
trans-Cadinene ether	1557	1557	0.36
β-Atlantol	1609	1608	0.49

[a]Experimental Linear retention index. [b]Relative Linear retention index to C_9–C_{22}*n*-alkanes on the DB-5 column taken from (Adams, 2007) for DB-5 capillary column in literature.

According to research, the yield and chemical composition of an EO is directly affected by various factors, including temperature, relative humidity, duration of insolation and wind conditions, cultivation practices, the harvest season of the plant, extraction method, geographical origin, and the complexity of the chemotype concept (Elbouny et al., 2022; Palá-Paúl et al., 2001; Selles et al., 2018). It should be noted that the refractive index of EOs varies primarily with the content of monoterpenes and oxygenated derivatives (Kanko et al., 2004), indicating that the preceding factors indirectly influence the physicochemical parameters of EOs.

Chemical composition

The chemical composition of the EOs of the *M. spicata*, *M. piperita,* and *M. pulegium* varieties revealed the presence of several chemotypes.

For the varieties of *M. spicata,* the first chemotype from the Akkari area is dominated by carvone/limonene, the second from the Sidi Ayach area is dominated by menthatriene/piperitone, and the third from the Ain Atiq area is dominated by piperitone. These major components have been cited in several studies for their antimicrobial, antispasmodic, anti-inflammatory and antioxidant activities (Abdolpour et al., 2007; Han et al., 2019; Pina et al., 2022; Santana et al., 2020). In Morocco, studies carried out on the chemical composition of *M. spicata* EOs that are harvested in the Settat region and the Sais valley reported that the oil was characterized by two main components: carvone and limonene. Previous studies in Algeria, Serbia, Greece, Tunisia, Iran, and India have shown that monoterpenes, notably carvone, limonene, and 1,8-cineole, were the main constituents of *M. spicata* EOs. All the previous studies cited reported a similar chemical composition of *M. spicata* EOs from the Akkari area (Bardaweel et al., 2018; Benomari et al., 2018; El Hassani, 2020; Fitsiou et al., 2016; Govindarajan et al., 2011; Shahbazi, 2015; Snoussi et al., 2015; Soković et al., 2009). The third chemotype, characterized by a high percentage of piperitone (77.84%), was reported for the first time in Morocco in the study, although it is a common Greek

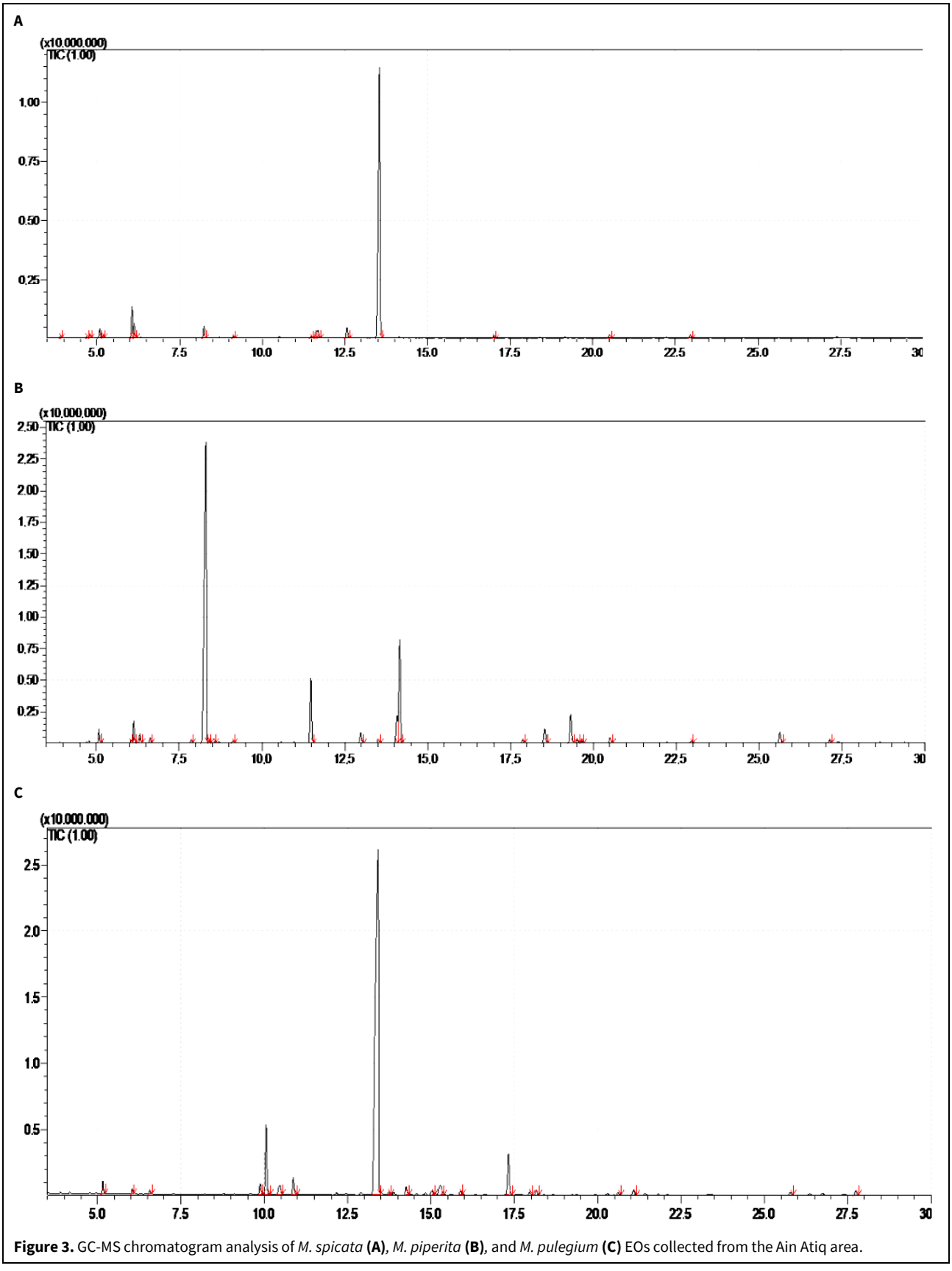

Figure 3. GC-MS chromatogram analysis of *M. spicata* **(A)**, *M. piperita* **(B)**, and *M. pulegium* **(C)** EOs collected from the Ain Atiq area.

Table 6. IC_{50} values of the EOs studied measured by various chemical tests.

Species	Origen	DPPH (mg/mL)	ABTS (mg/mL)	FRAP (mg/mL)
M. spicata	AK	27.63 ± 0.1616[a]	25.41 ± 0.4362[a]	5.36 ± 0.2573[a]
	SA	Inactive	17.93 ± 0.0658[b]	5.22 ± 0.3084[a]
	AA	16.44 ± 0.0392[b]	34.89 ± 2.4370[c]	8.62 ± 0.0873[b]
M. piperita	AK	25.15 ± 0.4733[c]	Inactive	68.39 ± 4.9820[c]
	SA	22.86 ± 0.1384[d]	28.75 ± 0.7127[d]	9.48 ± 0.0150[d]
	AA	58.04 ± 4.9856[e]	6.35 ± 0.3023[e]	35.87 ± 0.5147[e]
M. pulegium	AK	Inactive	5.02 ± 0.077[f]	7.28 ± 0.0096[f]
	SA	Inactive	1.73 ± 0.0271[g]	11.50 ± 0.2068[g]
	AA	Inactive	4.87 ± 0.2384[f]	12.59 ± 0.2546[h]
Quercetin	-	0.0054 ± 0.0001[f]	-	-
Catechin	-	-	-	0.013 ± 0.0025[i]
Ascorbic acid	-	-	0.0025 ± 0.0002[h]	-

Results were expressed as mean ± standard deviation (n = 3). Values in the same column with different superscript letters indicate significant differences ($p<0.05$). Ak: Akkari; SA: Sidi Ayach; AA: Ain Atiq

chemotype (Kofidis et al., 2004). Additionally, based on current knowledge, the second menthatriene/piperitone is a new chemotype found in the Sidi Ayach area and has not been described in the literature before.

Regarding *M. piperita*, the research showed the presence of three chemotypes depending on the areas studied. The Akkari area was dominated by linalool, the Sidi Ayach area by D-carvone, and the Ain Atiq area by 1,3,8-ρ-menthatriene. Studies carried out in Morocco, more specifically in the Taouanat and Middle Atlas regions, reported that the main components of Moroccan *M. piperita* EOs are menthol and menthone (Derwich et al., 2010; Marwa et al., 2007). These Moroccan results align with several studies (Camele et al., 2021; Likibi et al., 2015; Sustrikova and Salamon, 2004). In Algeria, *M. piperita* is essentially composed of menthol and menthone (Benabdallah et al., 2018), while in Iran, it is composed of α-terpinene and pipertitinone oxide (Yadegarinia et al., 2006). It is clear that the results diverge from those cited above. In fact, da Silva Ramos et al. (2017) found that *M. piperita* from Brazilian Macapa is predominantly composed of linalool, amounting to 51.8%, similar to Akkari's chemotype. Linalool and linalool-rich EOs have been cited by several researchers as having several biological activities, such as antimicrobial, anti-inflammatory, anticancer, and antioxidant properties (Kamatou and Viljoen, 2008).

The EOs of the *M. pulegium* samples are characterized by the presence of pulegone as the main component, with a concentration varying between 49.88% (Akkari area), 75.34% (Ain Atiq area), and 81.54% (Sidi Ayach area). Pulegone is an oxygenated monoterpene with well-known antimicrobial, anti-inflammatory, and antioxidant properties (Hariri et al., 2020; Rocha et al., 2019; Zougagh et al., 2019). Studies carried out in several regions of Morocco reported that pulegone is the main compound in *M. pulegium* (Bouyahya et al., 2017; Fadli et al., 2011; Lamiri et al., 2001). Luís et al. (2021) in Portugal and Hajlaoui et al. (2009) in Tunisia revealed that pulegone is the major component, with concentrations of 86.64% and 61.11%, respectively. However, previous studies on the chemical composition of pennyroyal mint have revealed the presence of other chemotypes. In Morocco, a new chemotype was reported rich in piperitone and piperitenone, with low levels of pulegone (Ait-Ouazzou et al., 2012). Beghidja et al. (2007) from Algeria found another one that is rich in monoterpenes such as α and β pinenes, camphene, sabinene, α-terpinene, and myrcene.

In fact, EOs compositions vary considerably due to a variety of extrinsic and intrinsic factors, such as ecological and climatic conditions, geographical location, season of collection, stage of development, phase of plant ontogeny, ecological conditions, harvesting methods, post-harvest treatment of plant material and the method of oil extraction along with chemotypic variations (Grulova et al., 2014; Hussain et al., 2010; Palá-Paúl et al., 2001; Selles et al., 2018). According to the results of the present study, it can be observed that the notion of chemotype, as well as the season of harvest, ecological conditions, and geographical location, played a major role in the fluctuation of the chemical composition of the samples studied.

Antioxidant capacity

Antioxidants play a vital role in human health, and they protect us from free radicals that may be responsible for a number of pathogenic and chronic diseases (Alsaraf et al., 2021). Mint species, in general, are considered to be safe natural antioxidants that can replace synthetic alternatives due to their metabolic richness (Fazili et al., 2020). It has been summarized that *Mentha's* high antioxidant capacity is always attributed to the presence of phenolic compounds such as thymol, carvacrol, as well as alcohols like citronellol and geraniol. Additionally, it exhibits particular richness in pulegone, menthol, menthone, piperitone, α-pinene, p-cymene, linalool, 1,8-cineole, limonene, borneol (Grulova et al., 2014; Hariri et al., 2020; Jagdale et al., 2015; Miguel, 2010). In this research, antioxidant capacity was measured using different methods to assess free radical scavenging potential (DPPH) and metal chelating potential (Fe^{2+} radicals) (FRAP) and ABTS.

In the DPPH test, only the EOs of *M. spicata* and *M. piperita* samples were able to reduce the stable DPPH radical to yellow DPPH-H. However, the best activity was observed in the Ain Atiq *M. spicata* sample (16.44 ± 0.0392 mg/mL). Compared with the high-performance antioxidant quercetin (0.0054 ± 0.0001 mg/mL), *M. spicata* has a moderate antioxidant potential. Several studies have demonstrated that *M. spicata* EOs are a potential source of the free radical scavenger DPPH (Fitsiou et al., 2016; Ouedrhiri et al., 2021; Saba and Anwar, 2018). Despite this, some studies have reported that *M. piperita* has a reduction potential of up to 50%, even surpassing *M. spicata* (Dorman et al., 2003; Mimica-Dukić et al., 2003). Furthermore, several studies present findings that differ from the observed results, indicating that *M. pulegium* exhibits excellent DPPH free radical scavenging activity. Kulisic et al. (2005) reported that the overall performance of an antioxidant is the result of the complex interaction between components, producing synergistic or antagonistic behavior. This finding aligns with the results obtained, as several major components (such as pulegone and linalool), known for their antioxidant capacity, were either inactive or displayed low activity.

The results of the ABTS test revealed that all the EOs studied were active in this test, except for the *M. piperita* sample from the Akkari area, which was inactive though active in DPPH. The best activity was observed in *M. pulegium*, more specifically from the Sidi Ayach area. This could be attributed to the presence of a large quantity of pulegone (81.54%). *M. piperita* from the Ain Atiq area also has a reasonable antioxidant capacity. *M. spicata* showed moderate activity, which is in line with the study by Bardaweel et al. (2018).

The reducing power assay (FRAP) is the ability of a natural antioxidant to donate an electron or hydrogen to form a more stable product (Shimada et al., 1992). All the EOs studied were capable of reducing the Fe^{3+}/ferric cyanide complex to the ferrous form. The best activity was noted regarding *M.spicata* in the Sidi Ayach area with an IC_{50} = 5.22 ± 0.3084 mg/mL, followed by *M. spicata* in the Akkari area with an IC_{50} = 5.36 ± 0.2573 mg/mL and *M. pulegium* in the Akkari area with an IC_{50} = 7.28 ± 0.0096 mg/mL. Bardaweel et al. (2018) have shown that *M. spicata* has great power to donate electrons to reactive free radicals, converting them into more stable non-reactive species and putting an end to the free radical chain reaction.

CONCLUSION

The chemical compositions of the essential oils of *M. spicata*, *M. piperita,* and *M. pulegium* collected in different geographical locations in Morocco were determined. The observed differences have been attributed to the existence of chemotypes. In Morocco, attention must be paid to commercial producers of EOs to avoid mixing species of *Mentha* during plant harvesting, especially from the different localities.

These species of *Mentha* genus, which are gaining increasing attention in the country, may potentially be used in the manufacture of pharmaceutical and oral-dental product preparations. They can also serve as natural antioxidants to replace synthetic products with toxic effects on human health. Additionally, it is suggested that the synergistic effect of these three *Mentha* varieties may result in a strong antioxidant capacity that surpasses the effect of synthetic products.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Contribution	Rayan A	Chadia O	Azzedine E	Abdellah M	Zakaria B	Abdelaziz E	Zahra BF	Otman E	Abdallah D	Lhousaine B
Concepts or ideas	x	x	x	x						
Design	x	x	x	x						
Definition of intellectual content	x	x	x	x						
Literature search	x	x	x	x						
Experimental studies	x	x	x	x	x	x	x	x		
Data acquisition	x				x	x		x		
Data analysis	x				x	x	x	x		
Statistical analysis										
Manuscript preparation	x	x	x						x	x
Manuscript editing	x	x	x		x	x			x	x
Manuscript review	x	x	x	x	x	x	x	x	x	x

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