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Antioxidant activity and chemical analysis of *Mentha spicata* cultivated from west northern region of Algeria by headspace solid phase micro-extraction and hydro-distillation

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ABSTRACT

The essential oil of a *Mentha spicata* L. (collected from Algeria) was extracted by hydrodistillation and solid phase micro-extraction (SPME). The oils have been studied by GC and GC-MS. Thirty seven compounds identified in the aerial parts oil extracted by hydrodistillation, the principal components being carvone (48.42%), eucalyptol (17.6%) and neoisodehydrocarveol acetate (11.7%). On the other hand, the oil extracted by SPME showed eucalyptol (55.1%) as the principal component with moderate amounts of carvone (7.2%), (Z)-dehydro-carvone (4.3%), cis-carveol (3.9%) and carvacrol (3.0%). In addition, it should be noted that 11 compounds identified only in the volatile fractions extracted using HS-SPME and not indentified in essential oils. Isolated essential oil was tested for radical-scavenging ability using the stable DPPH radical assay, which showed concentration-dependant antiradical activities, i.e. a percent of inhibition of 52.21% in the presence of 12.6 mg/mL.

KEYWORDS

Mentha spicata L.;
Lamiaceae;
Essential oil;
Chemical composition;
GC-MS;
SPME.

INTRODUCTION

Mentha is a well-known genus (family Lamiaceae) for medicinal and aromatic value. The genus *Mentha* includes 25–30 species that growin the temperate regions of Eurasia, Australia and South Africa[1]. These species showed considerable chemical diversity in essential oil composition.The genus is under cultivation from tropical to temperate climate of America, Europe, China, Brazil, India, etc[2]. *M. spicata* L. is a creeping rhizomatous, glabrous and perennial herb with a strong aromatic odor. The oil of *M. spicata* is rich in carvone and presents a characteristic spearmint odor[3]. The species has been found useful as digestive and gastrostimulant. Leaves are popularly used as teaflavouring agent, while herbalist uses whole plant as carminative[4]. The fresh and dried plants and their essential oils are widely used in food, cosmetic, confectionary, chewing

gum, toothpaste and pharmaceutical industries[5]. The essential oil of *M. spicata* showed strong insecticidal and mutagenic activity[6]. Different chemotypes are characterized by distinct smells and bioactivities, indicating different uses in aromatic and medicinal industries[7]. For instance, European enjoy carvone-scent, while Chinese prefer menthol-scent[8]. *Mentha* L. accessions from different geographical populationgenerally show numerous variations in the essential oil properties[8,9]. Several chemotypes are observed in *Mentha* from various locations. For example, four chemotypes of *M. spicata* are found in Greece, characterized by the dominant occurrence of linalool, carvone/dihydrocarvone, piperitone oxide/piperitetone oxide, and menthone/isomenthone/pulegone, respectively[8,9]. Although the genus *Mentha* has been widely studiedno investigations have been performed on the entire set of flavour volatiles of *M. spicata*. We have applied the headspace solid phase micro extraction (HS-SPME) and hydrodistillation extraction (HD) to extract the aroma volatiles of Algerian *M. spicata* before analysis. Briefly, the HS-SPME is used for the extraction of volatile compounds by the use of a fused silica fibre coated with different stationary phases. This is a common technique to evaluate the flavour compounds of various foods such as vegetables, fruits, juices, soft drinks or alcoholic beverages as recently reviewed from[10]. To the best of our knowledge, no studies have been published on the characterization of Algerian *Mentha spicata* aroma compounds with SPME.In this paper, we report on the essential oil composition of *M. spicata*, growing in Algeria, extracted by hydrodistillation (HD) and solid phase micro-extraction (SPME) followed by GC-MS analysis to obtain the most complete profile and get a better knowledge of components. The final objective of the present study is to assess the antioxidant activity of essential oil.

MATERIALS AND METHODS

Plant material and oil isolation

Mentha spicata L. was collected in Mai 2011 from Beloul 40km far from Saïda (868 (m), 34° 502 003 N, 0° 092 003 E) in the west northern region of Algeria. Voucher specimen was identified by Pr Nouri BENABADJI of Department of Biology - University of Tlemcen, Algeria and deposited in the institutional herbarium. The plants were cut at ground surface and taken to laboratory to extract essential oil. Chopped plants were used fresh for determination of essential oil content by hydrodistillation method using Clevenger-type apparatus at 60°C for 3 h. The essential oil was dried with anhydrous sodium sulphate, stored at 4°C and used for GC-MS analysis. The essential oil content (%) was determined on fresh weight basis as an average of three samples.

Gas chromatography analysis (GC)

GC analysis was performed using a Perkin-Elmer Clarus 600 GC apparatus (Walhton, MA, USA) equipped with a single injector and two flame ionization detectors (FID). The analysis was carried out using two fused silica capillary columns (60 m; 0.22 mm i.d.; film thickness 0.25 µm) with different stationary phases: Rtx-1 (polydimethylsiloxane) and Rtx-Wax (polyethylene glycol). The operating conditions were as follows: Injector and detector temperatures were maintained at 280°C. Helium was used as carrier gas (1mL/min), the injection volume was 0,1 µL, split ratio was adjusted at 1:80, the oven temperature was programmed from 60°C to 230°C at the rate of 2°C/min and then held isothermally at 230°C for 30 min.

Gas chromatography-mass spectrometry analysis (GC-MS)

The oils were investigated using a Perkin Elmer Turbo Mass quadrupole analyzer, directly coupled to a Perkin Elmer Autosystem XL equipped with two fused-silica capillary columns (60 m x 0.22 mm, film thickness 0.25 µm), Rtx-1 (polydimethylsiloxane) and Rtx-Wax (polyethylene glycol). Other GC conditions were the same as described above. Ion source temperature: 150°C; energy ionization: 70 eV; electron ionization mass spectra were acquired with a mass range of 35 – 350 Da; scan mass: 1s. Oil injected volume: 0.1 µL.

HS-SPME conditions

The aerial parts of *M. spicata* were cut roughly with scissors (1 - 2 cm long) before subjection to HS-SPME. The SPME device (Supelco) coated with divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, 30 µm) was used for extraction of the plant volatiles. Optimization of conditions was carried

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out using fresh aerialparts of the plant (1 g in a 20 mL vial) and based on the number and the sum of total peak areas measured on GC-FID. Temperature, equilibration time and extraction time were selected after nine experiments combining four temperatures (30, 50, 70 and 90°C), four equilibration times (20, 40, 60 and 80 min) and three extraction times (15, 30 and 45 min). After sampling, SPME fibre was inserted into the GC and GC-MS injection ports for desorption of volatile components (5 min), both using the splitless injection mode. Before sampling, each fibre was reconditioned for 5 min in the GC injection port at 260°C. HS-SPME and subsequent analyses were performed in triplicate.

Component identification

The identification of the oil components was performed by their retention indices (RI), authentic reference compounds, peak matching library search, as well as published mass spectra[11-14]. Retention indices werecalculated using an *n*-alkane series (C7–C25) under the same GC conditions as for the samples. The relative amount (%) of individual components of the oil is expressed as percent peak area relative to total peak area from the GC/FID analyses of the whole extracts.

Determination of antioxidant activity

The antioxidant activity of the samples was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging[15]. In test tubes, 0.25 mL of DPPH 0.8 mM in MeOH was added to accurately weighed aliquots of the extracts dissolved in 3.75 mL of MeOH, corresponding to concentration ranges of extract between 0.01 to 0.2 mg/mL. After mixing, the samples were maintained in the dark, at room temperature for 30 min. The absorbance at 517 nm was measured using a U.V/VIS Spectrophotometer (Optizen POP)and compared with a control without extract. A blank was prepared for each sample using methanol instead of the DPPH solution. Ascorbic acid was used as reference compound. Antioxidant activity was expressed as a percent inhibition of DPPH radical, and calculated from the equation:

Scavenging activity (%) = 100.

Abs control – Abs sample / Abs control

IC_{50} values were determined from the plotted graphs of scavenging activity against the concentration of the extracts. These values are defined as inhibitory concentration of the extract necessary to decrease the initial DPPH radical concentration by 50% and are expressed in mg/mL. Triplicate measurements were carried out.

RESULTS AND DISCUSSION

The aerial parts of *M. spicata* yielded 1,27±0,02% (w/w) (calculated on a dry weight basis) of a pale-greenish oil. The components identified from *M. spicata* oil, their retention indices and their percentage composition are summarized in TABLE 1 where all the com-

TABLE 1 : Volatile compounds identified in *M. spicata* using HS-SPME and hydrodistillation.

N°	Compounds[a]	RI_a[b]	RI_a[c]	RI_p[d]	HD	SPME	Identification[e]
1	3-methyl 1-Butanol	717	710	/	-	0.3	RI. MS. Ref.1
2	Hexanal	780	772	/	-	0.2	RI. MS. Ref.1
3	Heptanal	876	878	1187	tr	0.2	RI. MS
4	α-Thujene	922	924	1023	0.5	0.4	RI. MS
5	α- Pinene	931	932	/	-	2.6	RI. MS
6	6-methyl-3-Heptanone	935	937	1320	-	0.3	RI. MS. Ref.1
7	Camphene	943	945	1069	0.7	0.2	RI. MS
8	6-Methylhept-5-en-2-one	946	952	/	-	0.4	RI. MS
9	Sabinene	964	967	1122	-	1.6	RI. MS
10	β-pinene	970	972	1112	1.0	1.0	RI. MS
11	3-Octanol	986	981	1387	1.4	3.4	RI. MS
12	Myrcene	979	981	1160	0.9	0.5	RI. MS
13	3-methyl butylIsobutyrate	994	994	/	-	0.5	RI. MS. Ref.1
14	α-Phellandrene	997	1006	/	-	0.3	RI. MS
15	α-Terpinene	1008	1011	1180	-	1.5	RI. MS
16	*p*-Cymene	1011	1014	1268	0.1	-	RI. MS
17	Eucalyptol	1020	1025	1203	17.6	55.1	RI. MS

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N°	Compounds[a]	RI_a[b]	RI_a[c]	RI_p[d]	HD	SPME	Identification[e]
18	*(Z)*-β-Ocimene	1024	1027	1231	0.4	0.3	RI. MS
19	*(E)-β*-Ocimene	1034	1038	1239	0.2	-	RI. MS
20	*γ*-Terpinene	1047	1050	1244	0.3	0.7	RI. MS
21	trans-hydrate Sabinene	1051	1055	1457	1.5	0.8	RI. MS
22	Terpinolene	1078	1081	1282	0.1	0.3	RI. MS
23	Nonanal	1083	1084	1374	0.1	tr	RI. MS
24	Linalol	1081	1084	1554	0.1	-	RI. MS
25	3-octyl Acetate	1111	1110	1332	0.2	tr	RI. MS
26	Limonene-1,2-epoxyde-Z	1117	1117	/	-	0.1	RI. MS
27	(Z)-Linalol-oxide	1148	1143	/	-	0.2	RI. MS
28	Borneol	1148	1151	1687	0.2	0.1	RI. MS
29	Terpinene-4-ol	1161	1164	1598	0.9	2.5	RI. MS
30	(Z)-dehydro-Carvone	1173	1173	1616	1.3	4.3	RI. MS
31	α-Terpineol	1176	1178	1699	0.5	0.1	RI. MS
32	Neoiso-dehydro-Carveol	1178	1180	1743	11,7	3.5	RI. MS
33	*cis*-Carveol	1208	1206	1822	-	3.9	RI. MS
34	Carvone	1222	1223	1724	48.4	7.2	RI. MS
35	Pulegone	1222	1222	/	-	0.2	RI. MS
36	Carvotanacetone	1230	1224	/	-	0.7	RI. MS
37	Carvacrol	1282	1278	/	-	3.0	RI. MS
38	neodehydroCarvyleacetate	1311	1312	1665	1.1	tr	RI. MS
39	(E)-Jasmone	1364	1369	1889	0.3	0.2	RI. MS
40	*β*-Bourbonene	1385	1384	1511	1.2	0.2	RI. MS
41	*β*-Elemene	1388	1389	1587	0.8	-	RI. MS
42	*(E)*-β-Caryophyllene	1424	1419	1593	2.1	0.2	RI. MS
43	Germacrene D	1480	1477	1706	1.1	-	RI. MS
44	bicycle-Germacrene	1494	1491	1718	0.3	-	RI. MS
45	*β*-Bisabolene	1500	1500	1729	tr	-	RI. MS
46	*Trans*-Calamenene	1512	1510	1810	0.1	-	RI. MS
47	*δ*-Cadinene	1516	1515	1752	tr	-	RI. MS
48	α-Cadinene	1536	1531	1736	tr	-	RI. MS
49	*τ*-Cadinol	1632	1638	2195	0.2	-	RI. MS
	Total (%)				95.3	97.0	
	Hydrocarbon compounds				9.8	10.2	
	Oxygenated compounds				85.5	87.2	
	Hydrocarbon monoterpenes				4.2	9.4	
	Hydrocarbon sesquiterpenes				5.6	0.4	
	Oxygenated monoterpenes				83.6	81.9	
	Oxygenated sesquiterpenes				0.2	-	
	Aliphaticcompounds				1.7	5.3	

[a] Order of elution is given on apolar column (Rtx-1), [b] Retention indices of literature on the apolar column (RIa) reported from König*et al.*, 2001,[c] Retention indices on the apolar Rtx-1 column (RIa), [d] Retention indices on the polar Rtx-Wax column (RIp), [e] RI: Retention Indices; MS: Mass Spectrometry in electronic impact mode; Ref1,: compounds identified from literature data König*et al.*, 2001.

pounds are arranged in order of their elution on the RTX-1 column. The principal compounds (>1.0%)

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appear in bold face. Two different columns, a polar (RTX-1) and a non-polar (RTX-Was), have been used in the GC-MS analysis to identify the majority of the components. An analysis of the essential oil of *M. spicata* harvested in west northern region of Algeria identified 37 components, which accounted for 95.3% of the total number. Their retention indices and relative percentages are shown in TABLE 1. Among these, 24 monoterpenes, 10 sesquiterpenes and 4 aliphatic compounds were identified. All components were identified by comparison of their EI-MS and GC-retention indices with those of our laboratory-produced "Arômes" library, with the exception of four components that were identified by comparison with spectral data and retention indices from the literature. The oil extracted by hydrodistillation was characterized by a large amount of monoterpenes (87.8%) made up of oxygenated monoterpenes (83.6%) and hydrocarbon monoterpenes (4.2%). Hydrocarbon compounds represented only 10.28% of the oil, most of them being oxygenated (32.7%). The principal compounds were found to be carvone (48.42%), eucalyptol (17.6%) and neoisodehydrocarveol acetate (11.7%). Other representative compounds were identified as (E)-β-Caryophyllene (2.1%), trans-hydrate sabinene (1.5%), 3-Octanol (1.4%), (Z)-dehydrocarvone (1.3%), β-bourbonene (1.2%), neo isodehydrocarvyle acetate (1.1%), germacrene D (1.1%) and β-pinene (1.0%)were minor constituents of the oil.

The oil vapour adsorbed by headspace SPME showed higher amounts of monoterpenes (91.3%) than sesquiterpenes (0.4%). As in the hydrodistilled oil, oxygenated monoterpenes (81.9%) were found in higher amounts than the hydrocarbons (9.4%). However, sesquiterpenes are represented only by hydrocarbon sesquiterpenes (0.4%). Other characteristic compounds of the oil were identified as cis-carveol (3.9%), carvacrol (3.0%), α-pinene (2.6%), terpinene-4-ol (2.5%), sabinene (1.6%) and α-terpinene (1.5%). Quantitative but not qualitative differences have been found in the chemical composition of both analysed samples depending of the extraction method. Carvone (7.2-48.4%) was found as the principal component of this species but it was found in greater concentrations in the essential oil than in the SPME extracts. Inversely, eucalyptol (17.6–55.1%) was found in greater concentrations in the SPME extracts than in the hydrodistillation ones. The chemical differences observed between both the essential oils and the volatile fractions extracted using HD and SPME, respectively, can be explained by the fact that the first technique is based on the liquid quasitotal extraction of plant volatiles and the latter technique is controlled by a solid/gas equilibrium step. During hydrodistillation, the most volatile compounds and water-soluble compounds are lost in the gaseous phase and in the hydrolate, respectively, whereas, with HS extraction, it is the fiber affinity of each compound that monitors the sampling of the volatiles. As a consequence, it should be noted that 11 compounds (1, 2, 5, 8, 1, 14, 26, 27, 35, 36 and 37) were identified only in the volatile fractions extracted using HS-SPME. As stated above the oil from *M. spicata* is characterized by high amount of eucalyptol. This compound does not usually appear as dominant in oils of other *Mentha* species.

Radical scavenging activity

DPPH method has been widely used in the determination of the antiracial activity of single compound as well as different plant essential oils. This method is based on the reduction of alcoholic DPPH solutions in the presence of a hydrogen donating antioxidant. The method was used to evaluate the antioxidant properties of the *M. spicata* in comparison with the synthetic antioxidant (ascorbic acid). As shown in TABLE 2, this

TABLE 2 : Antioxidant activity of essential oil of *M. spicata* using DPPH testing method.

Sample	Antioxidantactivity					
Oil	Extract concentration (mg/mL)	0.2	4.2	6.3	12.6	
	Scavengingeffect on DPPH (%)	16.32	32.72	42.64	52.21	
	DPPH IC_{50} (mg/mL)					10.62
Ascorbicacid	Extract concentration (mg/mL)	0.04	0.05	0.06	0.08	
	Scavengingeffect on DPPH (%)	39.40	51.03	68.57	97.84	
	DPPH IC_{50} (mg/mL)					0.048

essential oil showed concentration-dependant antiradical activities. These results show a percent of inhibition of 52.21% in the presence of 12.6 mg/mL. The antioxidant activity of the essential oil can be also evaluated by the determination of the IC_{50} values corresponding to the amount of extract required to scavenge 50% of DPPH radicals present in the reaction mixture. High IC_{50} values indicate low antioxidant activity. Assessed sample was able to reduce the stable violet DPPH radical to the yellow DPPH, reaching 50% of reduction with IC_{50} value of 10.62 mg/mL. This IC_{50} values was less than the IC_{50} obtained for ascorbic acid (0.048 mg/mL) used as positive control. In addition, the antioxidant activity of our essential oil is probably due to the presence of great amount of oxygenated compounds (85.5%).

CONCLUSIONS

In this work, we were able to show that the chemical composition of *M. spicata* essential oil from west-Northern of Algeria is dominated by carvone (7.2-48.4%) and eucalyptol (17.6–55.1%). So, this study demonstrates that HD and HS-SPME modes could be complimentary extraction techniques in order to obtain the complete characterization of plant volatiles. Other hand, essential oil showed moderate antioxidant activity.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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REFERENCES

[1] H.J.Dorman, M.Kosar, K.Kahlos, Y.Holm, R.Hiltunen; J.Agric.Food Chem., **51**, 4563 **(2003)**.
[2] S.P.S.Khanuja; Employ contract farming to boost area under cultivation for essential oil bearing crops. In Business enabling of aromatic plants and products, 21e22 November 2007 at HRDI Dehradun. Chemical weekly, 25 December, 180e184 **(2007)**.
[3] L.Jirovetz, G.Buchbauer, M.Shabi, M.B.Ngassoum; Perfum.Flav., **27**, 16 **(2002)**.
[4] Y.M.Yonis, S.M.Beshir; J.Essent.Oil Res., **16**, 539 **(2004)**.
[5] B.M.Lawrence; Mint: The Genus *Mentha*. CRC Press, Boca Raton, FL, **(2006)**.
[6] R.Karousou, M.Balta, E.Hanlidou, L.Kokkini; Mints, smells and traditional uses in Thessaloniki (Greece) and other Mediterranean countries. J.Ethnopharmacol., **109**, 248 **(2007)**.
[7] D.Zhao, Y.Wen Xu, Guo.Li Yang, M.Amjad, Wei Wu.Husaini; Ind.Crop.Prod., **42**, 251 **(2013)**.
[8] A.Orav, A.Raal, E.Arak; Proc.Est.Acad.Sci.Chem., **53**, 174 **(2004)**.
[9] G.Kofidis, A.Bosabalidis, S.Kokkini; J.Essent.Oil Res., **16**, 469 **(2004)**.
[10] H.Kataoka, H.L.Lord, J.Pawliszyn; Journal of Chromatography A., **880**, 35 **(2000)**.
[11] Arômes library of Laboratory of Chemistry of Natural Products.University of Corse, Corte, France.UMR CNRS 6134, **(1987–2011)**.
[12] W.A.König, D.H.Hochmuth, D.Joulain; Terpenoids and related constituents of essential oils. Library of Mass Finder 2.1 University of Hamburg, Institute of Organic Chemistry, Hamburg, Germany, **(2001)**.
[13] R.P.Adams; Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry, 4th edition. Allured Publishing Corporation, Carol Stream, IL, USA, **(2007)**.
[14] NIST/EPA/NIH, NIST/EPA/NIH Mass Spectral Database, Version 2.1. Perkin Elmer Instrument LLC, Copyright, **(2000)**.
[15] O.P.Sharma, T.K.Bhat; Food Chem., **113(4)**, 1202 **(2009)**.