Spearmint (Mentha spicata L) essential oil cultivated in Algerian Sahara (Ouargla): Chemical composition and comparison with other regions of Algeria and Mediterranean area

Mekhelfi Tarak[*1], Zaoui–Djelloul Daouadji Manel[1], Belfar Mohammed Lakhder[1], Mekhloufi Nour El Houda[2]

[1]VPRS Laboratory. Department of chemistry. FMSM Faculty. University of KASDI Merbah, Ouargla, 30000. Algeria

[2]Department of chemistry. FMSM Faculty. University of KASDI Merbah Ouargla, 30000. Algeria

[*]Corresponding author: MEKHELFI Tarak, Affiliation: VPRS Laboratory, Department of chemistry, FMSM Faculty, University of KASDI Merbah, Ouargla, 30000, Algeria, tarakmekhelfi@gmail.com

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Abstract

The aim of this work is to study the chemical composition of Algerian Mentha spicata essential oils cultivated in the Algerian Sahara (region of Ouargla). On the other hand, a comparison of Mentha spicata essential oils studied with others growing in different regions in Algeria as well as in the Mediterranean area (Percentage of major components ≥ 5%) and in the world (Percentage of major components ≥ 1%). The essential oil of Spearmint (Mentha spicata) cultivated in the Algerian Sahara in the region of Ouargla was extracted by hydrodistillation. Seventy-three compounds were identified in the aerial parts of Mentha spicata. The principal components are : D-carvone (28.24%), eucalyptol (19.63%), D-limonene (09.20%), beta.-Myrcene (07.36%), and Cyclohexanone, 2-methyl-5-(1-methyl ethenyl) (05.33%).

Keywords: Mentha spicata L, essential oils, hydrodistillation, Eucalyptol, GC-MS.

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INTRODUCTION

Throughout human history, herbal treatments have been used to cure a variety of infectious diseases. Plant-based treatments still play a significant role in primary healthcare today, especially

in developing countries [1] .According to Nikbakht and Kafi [2], medicinal plants include plant components such as leaf, root, flower, and seed that are used to make chemical compounds and medications for humans and animals. Very potent natural physiologically active substances can be found in the essential oils and extracts of several species of edible and medicinal plants, herbs, and spices. An additional intrinsic factor to improve the safety and shelf life of foods may be the use of essential oils as antibacterial agents in food systems [3].

Algeria is one of the richest countries of the Mediterranean area in phylogenetic resources of aromatic and medicinal interest because of the diversity of its bioclimatic stages. There are more than 300 species of therapeutic or aromatic plants use among the 3,150 plant species that our country has. Most of the studies that were interested in the study of medicinal and aromatic plants in Algeria paid great attention to the academic aspect only, and did not highlight the importance of these plants in the revival of the economy as a renewable wealth. Algeria is considered as one of the most important importers of Egyptian medicinal and aromatic plants in spite of its capacity to plant these plants and to realize their export [4].

At this crucial stage in the history of Algeria, all our energies must be directed towards building a real renaissance for Algeria in all fields, especially for strategic fields and projects, of which the project for the production of medicinal raw materials is one of the most important, but in the first stage we will work on developing the division of extracting essential oils from medicinal and aromatic plants.

The genus Mentha, according to the most recent taxonomic, consists of 61 species that are divided into four sections: Pulegium, Tubulosae, Eriodontes, and Mentha [48]. This last is one of the more significant members of the Lamiaceae family; this genus is represented in the flora of Algeria by five important species: Mentha aquatica L., Mentha pulegium L., Mentha spicata L., and Mentha longifolia (L.) [5]. Mentha species are frequently used by locals in El-Tarf region (Algeria), located in the far northeastern corner of Algeria, as a spices, flavoring, or herb in culinary dishes. They are also used for their medicinal capabilities as a carminative, sedative, antispasmodic, and are well recognized for treating stomach pain [6].

Mentha spicata is widely used in phytomedicine as an antifungal, diuretic, carminative, and antioxidant agent as well as for the treatment of colds, the flu, hemorrhoids, respiratory tract issues, and stomachaches. It also offers a number of therapeutic characteristics and health advantages. It is interesting to note that oxygenated monoterpenes, which are abundant in spearmint essential oils (SEOs), have been linked to antibacterial action [7, 8]. It is also a genuine treatment option that is nontoxic for treating patients' emesis and nausea brought on by chemotherapy [9]. When making tea, the dry or fresh spearmint leaf is specifically included. The decoction of spearmint leaves is used to treat biliary problems, menstrual cramps, stomach pain, constipation, gingivitis, and ondotalgies [10] .According to morphological, cytological and biochemical data [11,12,13], M. spicata L. was described as the fertile hybrid resulting from the

M. suaveolens Ehrh cross. x M. longifolia (L.) Huds., two closely related and interfertile diploid species.

In this study, we aim to determine the chemical composition of Algerian Mentha spicata essential oils cultivated in the Algerian Sahara (region of Ouargla) followed by a comparison with that growth in other regions of Algeria as well as in the Mediterranean area (Percentage of major components ≥ 5%) and the other one of the world (Percentage of major components ≥ 1%).

2. Experimental part

2.1. Plant material

The aerial parts of cultivated Mentha spicata L., were collected in May 2022, from Ouargla (Algerian Septentrional Sahara). A voucher specimen was deposited at the herbarium of the University of KASDI Merbah - Ouargla, Algeria.

2.2. Extraction

The hydrodistillation of fresh aerial parts using a Clenvenger-style apparatus. (300 g) of Mentha spicata, for 3 hours, yielded 1.5% of a pale yellowish essential oil.

2.3. Gas chromatography GC

analyses Gas chromatography GC were performed using the following data analytique : GC Program (GCMS-TQ8040 NX) , column BPX-5 (Length 30 m; 0.25 mm I.D.; df = 0.25 µm) The oven temperature was programmed as isothermal at 60°C for 5 min, then Column Flow :0.50 mL/min.

2.4. Gas chromatography-Mass spectrometry

GCMS-TQ8040 NX Triple Quad GC/MS/MS Featuring Smart Productivity for high-efficiency sample throughput, Smart Operation for quick and easy method development, and Smart Performance for low detection limits and Scan/MRM, the GCMS-TQ8040 NX provides accurate, cost-effective and user-friendly triple quadrupole GC-MS/MS operation for routine analytical work. Among Features : i Enables analysis of +400 compounds in a single MRM run without sacrificing sensitivity or selectivity, MRM Optimization Tool – seamlessly selects and optimizes MRM transitions for multiple compounds in a single sequence, High-speed MRM analysis up to 800 transitions per second. The MS operating parameters were as follows: Injection Mode: Split Flow Control Mode: Column Flow, Pressure: 10.0 kPa, Total Flow: 28.5 mL/min, Column Flow: 0.50 mL/min, Linear Velocity: 26.0 cm/sec, Purge Flow: 3.0 mL/min, Split Ratio: 50.0. 3.

RESULTS AND DISCUSSION

The oil yield of M. spicata was 1.5%. Our results are in agreement with those of Laggoune et al.

[14] .These authors found that the yield of essential oil from M. spicata cultivated at Ghardaïa region (Algerian Septentrional Sahara) is 1.8%. However, the extraction yield of aerial parts of this species that was collected of Bangladesh of M. spicata was 0.4% [16] and in India was 0.3% [17]. The extraction yield found by Boukhbti et al. [18] from leaves of spearmint harvested from another location from Algeria (Setif) was 0.9%. The aerial parts of this species that was collected from Saida in the west northern region of Algeria yielded 1.3% [19], another study reported that the yield of essential oil from of M. spicata cultivated in Algerian Saharan Atlas was 1.04% [15]. We can conclude from this yield that the percentage of essential oil extracted from the M. spicata plant grown in the Algerian desert (especially in Ouargla and Ghardaïa) is greater than the percentage of the essential oil extracted from the wild M. spicata plant.

Hussain et al. [20] stated that the largest essential oil yield for Mentha species was observed in the summer when the plants were in full bloom with a yield about 1.2%, as compared to the winter with 0.95% in yield, when the plants had reached the end of their growing season.

The essential oil was extracted by the hydrodistillation of the dried aerial parts of M. spicata cultivated in the Algerian Sahara (Ouargla) and was analyzed by GC-MS. The yield of the oil was 1.50 mL per 100 g plant material, with a pale-yellow color and persistent aromatic-spicy odor. As shown in Table 1, analysis of the essential oil resulted in identification of 73 compounds, representing 100 % of the total oil, The major components of the oil were carvone (28.24%), limonene (09.20%), beta.-Myrcene (7.36%), Cyclohexanone (5.33%), Eucalyptol(19.63%), (-)-8-p-Menthen-2-yl, acetate, trans D (2.62%) and β-pinene (2.81%).

The character component found in this oil is Eucalyptol with high percentage (19.63%). It has many biological activity for this component; a therapeutic potency against bronchial asthma, COPD, gastric inflammation, sinusitis and even several neurodegenerative disorders like Alzheimer's because of its anti-inflammatory activity Eucalyptol depresses CNS and shows anti-nociceptive property, it enhances blood circulation, causes vasodilation and also bronchodilation.

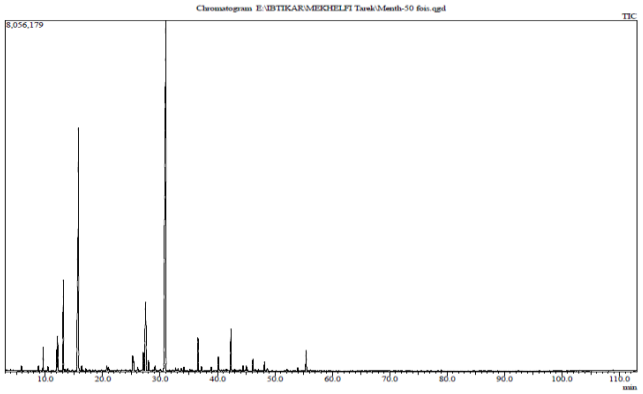

Figure 1. Chromatographic profile of M. spicata L. essential oil from Algeria. Compounds are numbered as listed in Table 1.

Peak #	R.Time	Area	Area %	Height	Height %	Similarity	Name
1	3.020	22840	0.01	20545	0.07	78	4-Nonene, 5-butyl-
2	3.981	106055	0.04	29605	0.10	95	Butanoic acid, 2-methyl-, methyl ester
3	5.770	109340	0.04	26517	0.09	90	Butanoic acid, 2-methyl-, ethyl ester
4	5.862	448879	0.18	105429	0.37	96	2-Hexenal
5	8.817	579479	0.23	112940	0.40	96	5,5-Dimethyl-1-vinylbicyclo[2.1.1]hexane
6	9.635	2900733	1.18	543554	1.91	95	2-Pinene
7	10.46	542364	0.22	100311	0.35	94	Camphene
8	11.98	2697750	1.09	100311	0.35	96	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-
9	12.15	4605133	1.87	479454	1.69	94	2(10)-Pinene
10	13.14	11846821	4.80	799221	2.81	95	.beta.-Myrcene
11	13.42	326672	0.13	2091227	7.36	93	3-Octanol
12	13.90	289578	0.12	48597	0.17	78	Myrtanyl 2-Methylbutyrate
13	15.63	16291742	6.60	39290	0.14	92	D-Limonene
14	15.76	37021433	15.01	2612074	9.20	90	Eucalyptol
15	16.34	660763	0.27	5575167	19.63	97	(3E)-3,7-dimethylocta-1,3,7-triene
16	17.05	307994	0.12	124103	0.44	96	1,3,6-Octatriene, 3,7-dimethyl-, (E)-
17	17.72	105890	0.04	56647	0.20	94	gamma.-Terpinene
18	18.28	123970	0.05	19018	0.07	94	5-Isopropyl-2-methylbicyclo[3.1.0]hexan-2-ol
19	19.82	182267	0.07	20406	0.07	91	Cyclohexene, 1-methyl-4-(1-methylethylidene)-
20	20.70	1021692	0.41	31107	0.11	96	Linalool

21	21.03	424258	0.17	108198	0.38	89	Butanoic acid, 2-methyl-, 2-methylbutyl ester
22	22.53	168287	0.07	68839	0.24	82	2,7-Octadiene-1,6-diol, 2,6-dimethyl-
23	25.25	2342906	0.95	27490	0.10	91	Borneol
24	25.37	1415474	0.57	340872	1.20	91	Cyclohexanemethanol, .alpha.,.alpha.-dimethyl-4-methylene-
25	26.09	554575	0.22	244253	0.86	92	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-
26	26.89	120044	0.05	84095	0.30	87	5-Hepten-1-ol, 2-ethenyl-6-methyl-
27	27.08	2806470	1.14	20731	0.07	94	alpha.-Terpineol
28	27.48	16595338	6.73	410566	1.45	94	Cyclohexanone, 2-methyl-5-(1-methylethenyl)-, (2S-cis)-
29	27.99	1502244	0.61	1514870	5.33	96	Cyclohexanone, 2-methyl-5-(1-methylethenyl)-, trans-
30	28.77	138320	0.06	236452	0.83	94	Acetic acid, octyl ester
31	29.13	1084507	0.44	23650	0.08	94	2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, cis-
32	30.05	185587	0.08	118559	0.42	84	Carveol
33	30.99	108382632	43.94	25239	0.09	95	D-Carvone
34	31.67	126968	0.05	8020650	28.24	95	Geraniol
35	32.22	287431	0.12	17310	0.06	90	3-hydroxy-2-methyl-5-(prop-1-en-2-yl)cyclohexanone
36	32.70	647037	0.26	30521	0.11	95	(2E)-3,7-Dimethyl-2,6-octadienal
37	33.13	412923	0.17	67001	0.24	91	Piperitenone oxide
38	33.71	526370	0.21	52723	0.19	95	1,3,3-Trimethylbicyclo[2.2.1]hept-2-yl acetate
39	34.15	603799	0.24	53527	0.19	88	2H-1-Benzopyran, 3,4,4a,5,6,8a-hexahydro-2,5,5,8a-tetramethyl-,

							(2.alpha.,4a
40	35.20	290686	0.12	91466	0.32	92	(-)-8-p-Menthen-2-yl, acetate, trans
41	35.55	175688	0.07	44489	0.16	85	Acetic acid, nonyl ester
42	36.60	4547657	1.84	25229	0.09	94	(-)-8-p-Menthen-2-yl, acetate, trans
43	37.22	688279	0.28	743522	2.62	86	2-Cyclohexen-1-one, 3-methyl-6-(1-methylethylidene)-
44	38.94	861883	0.35	97410	0.34	95	Rotundifolone
45	39.58	167974	0.07	89020	0.31	86	Copaene
46	40.16	2296303	0.93	12746	0.04	96	Cyclobuta[1,2:3,4]dicyclopentene, decahydro-3a-methyl-6-methylene-1-(1-me
47	40.67	157237	0.06	305479	1.08	92	1-Methyl-1-ethenyl-2,4-bis(1'-methylethenyl)cyclohexane
48	41.05	225706	0.09	21043	0.07	90	2-Cyclopenten-1-one, 3-methyl-2-(2-pentenyl)-, (Z)-
49	41.52	324571	0.13	19978	0.07	83	1H-Cycloprop[e]azulene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,4,7-tetramethyl-, [
50	41.75	218483	0.09	33893	0.12	91	1H-Cyclopropa[a]naphthalene, 1a,2,3,3a,4,5,6,7b-octahydro-1,1,3a,7-tetramet
51	42.33	6098145	2.47	27814	0.10	95	Caryophyllene
52	42.95	269570	0.11	923933	3.25	94	(1R,2S,6S,7S,8S)-8-Isopropyl-1-methyl-3-methylenetricyclo[4.4.0.02,7]decan
53	43.17	224353	0.09	36426	0.13	77	9,10-

							Dimethyltricyclo[4.2.1.1(2,5)]decane-9,10-diol
54	43.89	296200	0.12	32810	0.12	92	(S,1Z,6Z)-8-Isopropyl-1-methyl-5-methylenecyclodeca-1,6-diene
55	44.44	884862	0.36	27129	0.10	97	alpha.-Humulene
56	44.83	171413	0.07	116927	0.41	92	(E)-.beta.-Famesene
57	45.04	831315	0.34	26054	0.09	94	cis-Muurola-4(15),5-diene
58	45.28	203621	0.08	113377	0.40	89	(-)-Germacrene D
59	46.15	1810867	0.73	26545	0.09	97	(-)-Germacrene D
60	46.56	284334	0.12	266220	0.94	93	Butanoic acid, 2-methyl-, 2-phenylethyl ester
61	47.12	246380	0.10	36011	0.13	91.	Di-epi-.alpha.-cedrene
62	48.17	1411810	0.57	35520	0.13	96	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methyle
63	48.65	635119	0.26	216596	0.76	94	94 cis-Calamenene
64	49.56	117921	0.05	54269	0.19	93	93 Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, [1S-(1
65	51.71	78318	0.03	17651	0.06	87	87 (2E,4S,7E)-4-Isopropyl-1,7-

							dimethylcyclodeca-2,7-dienol
66	52.10	345424	0.14	9548	0.03	92	92 5-Oxatricyclo[8.2.0.04,6]dodecane, 4,12,12-trimethyl-9-methylene-, (1R,4R,6
67	53.30	91974	0.04	45494	0.16	84	84 Cycloheptane, 4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl-
68	53.99	477953	0.19	11076	0.04	95	95 4a(2H)-Naphthalenol, 1,3,4,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)
69	54.53	181814	0.07	69634	0.25	84	84 .alpha.-Patchoulene
70	55.44	3033884	1.23	10688	0.04	95	95 .tau.-Cadinol
71	56.19	172394	0.07	454835	1.60	91	91 .alpha.-Cadinol
72	58.11	211502	0.09	25835	0.09	91	91 Cyclohexanol, 3-ethenyl-3-methyl-2-(1-methylethenyl)-6-(1-methylethyl)-, [1
73	78.93	121699	0.05	21433	0.08	94	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-

Table 1. Chemical composition of M. spicata L. essential oil from Algeria.

If one look on table 2, one can conclude that the major component of Mentha spicata is carvone (48.4%- 59.4%) except for Ouargla region that has a percentage of 28.24% in carvone. The most component found in the mentha spicata essential oil of algeria is : Limonene(5.80%-24.99%), β-pinene(1.1%- 2.81%),1,8 – Cineole (3.8%- 15.32%), B-caryophyllene (2.1%-3.4%), β-myrcene (0.8%- 24.99%), Eucalyptol was found just in two essential oil and with great percentage(17.6% and 19.63%). In our study, we identified largest number of component (73 components).

Major components (≥ 1%)	Ouargla (our study)	Setif [21]	Laghouat [22]	Tlemcen 01 [23]	Laghouat [24]	Tipaza [25]	Ghardaia [14]	Tlemcen 02 [26]	Bejaya [27]
α-pinene	1.91	-	-				1.30	-	-
β-pinene	2.81	-	1.1		1.1	1.97	3.23	-	1.3
β-myrcene	7.36	-	-				5.11	-	20.8
Limonene	9.20	6.1	16.1	21.9	16.1	24.99	5.80	-	-
tau.-Cadinol	1.60	-	-					-	-
1,8 – Cineole	19.63	3.8	8.7		8.7	7.22	15.32	17.6	5.4
α-Terpineol	1.45	1.9	-						
(-)-8-p-Menthen-2-yl, acetate, trans	2.62	-	-						
Borneol	1.20	-	-						
β-bourbonène	2.70	-	-			1.87		1.2	1.4
Germacrène	4.66	-	2.1						3.4
Carvone	28.24	59.4	49.5	54.1	49.5	52.60	44.6	48.4	48.5
Terpinen – 4 – ol	-	-	1.5	1.3	1.5	1.22		-	1.5
Pulegone	-	-	-					-	-
t-Cadinol	-	-	-				3.28	-	-
β-phellandrene	-	-	-				1.96	-	-
cis-Dihydrocarvone	-	1.55	3.9				8.85		
Menthone	-	-	-						

B-caryophyllene	3.25	2.9	2.7		2.7		1.53	2.1	3.4
Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	1.69	-						-	-
Neoiso-dehydro-Carveol	-	-	-					11,7	1.3
trans-hydrate Sabinene	-	-	-					1.5	-
3-Octanol	-	-	-					1.4	-
β-phellandrene	-	-	-				1.96		

Table 2 Percentage of major components (≥ 1%) of essential oil of Mentha spicata growing in algeria.

From table 3, representing the major components (≥5%) of the studied essential oils of M. spicata, growing in the Mediterranean area, one can conclude that the couple eucalyptol/ carvone is a chemotype of M. spicata collected from Algeria (Our study), Turkey [32], Morocco [33] and Tunisia [28], the highest content (40.8, 17.0%) is found in Tunisia [28]. When checking table 3, we find that each essential oil contain eucalyptol the yield of caravone low than usual especially in Algeria (Ouargla) and Tunisia. The second chemotype in the essential oils of M. spicata is limonene/ carvone, it is also chemotype of M. spicata collected from Portugal [34], Egypt 01 [35], Tunisia [28] and Greece [30]. The highest content (68.58, 16.4%) is found in Egypt 01[35] whereas the lowest content (40.8, 20.8%) was found in plants collected from Tunisia [28]. The essential oil of the species collected from Morocco [33] is mainly characterized by carvone (29.0%) and trans-carveol (14.0%) [16]. Pulegone (26.71-29.56%), piperitone (22.17-28.16%) dominated in the composition of essential oil of M. spicata from Turkey followed by 1, 8-cineole (6.84-7.76%) and trans-β-caryophyllene (5.2-8.0%) [32].

Compounds	**Algeria (Ouargla)**	**Greece [31]**	**Turkey [32]**	**Morocco [33]**	**Portugal [34]**	**Egypt01 [35]**	**Egypt02 [36]**	**Tunisia [28]**	**Italy [29]**	**Greece [30]**
Limonene	9.20	-	-	-	20.1	16.4		20.8	-	20.8

1,8-Cineole (Eucalyptol)	19.63	14.5	6.84-7.76	7.30	-	-	19.55	17.0	-	-
trans-Carveol	-	-	-	14.0	-	-	-	-	-	-
Pulegone	-	-	26.7-29.5	-	-			-	34.1	
Carvone	28.24	-	-	29.0	41.1	68.58		40.8	-	67.1
Piperitone	-	-	22.1-28.1	-	-	-		-	-	-
Piperitenone oxide	-	35.7	-	-	-	-	-	-	32.9	-
Copaene	-	-	-	-	5.6	-	-	-	-	-
trans-β-Caryophyllene	-	-	5.2-8.0	-	-	-	-	-	-	-
β-myrcene	7.36	-	-	-	-	-	-	-	-	-
α-Pinene	-	-	-	-	-	-	5.26	-	-	-
4-Terpineol	-	-	-	-	-	-	6.28	-	-	-
α -Terpineol	-	-	-	-	-	-	5.38	-	-	-
trans-p-Menthan-8-ol, acetate	-	-	-	-	-	-	6.42	-	-	-
trans-Cadina-1,4-diene		6.4								
Spathulenol		5.2								

Table 3: Percentage of major components (≥ 5%) of essential oil of Mentha spicata growing in the Mediterranean area.

One can see from the table 4, that M. spicata growing in Ouargla (Our study) contain the biggest number of chemotype ≥ 5% (Carvone, eucalyptol, Limonene and β-myrcene). In other studies the major components of M. spicata growing in most countries are the couple carvone/limonene, which is also a chemotype of most reported essential oils [38-39;41-42; 45-47], its highest yield was (76.65; 22.31%) growing in both North-West Himalayan, and India

regions [41]. However, menthone (21.9%) was a chemotype of M. spicata, collected from Serbia [19]. In addition, β-bourbonene (11.23%), α-humulene (0.1-29.9%) and pulegone (72.1%), were mainly exclusive from Iran [38], Uttarakhand, India [40] and Karnataka, India [43] respectively. In addition, menthone (21.9%) was a chemotype of M. spicata collected from Serbia [37]. In another part, piperitenone oxide (52.3%) was mainly exclusive from Cuba [30] whereas cis- carveol (21.3%) was the major compound collected from Tamilnadu, India [39]. Furthermore, eucalyptol was a chemotype of M. spicata, collected from Ouargla (Algeria).

Compounds	Algeria (Ouargla)	Serbia [37]	Iran [38]	India 01[39]	India 02[40]	India 03[41]	India04[42]	India05[43]	USA [44]	China [45]	Yemen [46]	Pakistan [47]
Limonene	9.20	-	11.5	11.3	-	9.5-22.3	10.1	-	-	18.19	18.2	5.3
Menthone	-	21.9	-	-	-	-	-	18.6	-	-	-	-
cis-Carveol	-	-	-	21..3	-	-	-	-	-		-	24.3
Pulegone	-	-	-		-	-		72.1	-	-	-	-
Carvone	28.24	69.5	78.76	48.6	15.3-68.5	49.6-76.6	57.1		40.12	65.33	65.3	51.7
Piperitenone oxide	-	-	-	-	24.0-79.2	-	-	-	-	-	-	-
β-Bourbonene	-	-	11.23	-	-	-	-	-	-	-	-	-
α-Humulene	-	-	-	-	0.1-29.9	-	-	-	-	-	-	-
1,8-Cineole (eucalyptol)	19.63	-	-	-	-	-	-	-	-	-	-	-
β-myrcene	7.36	-	-	-	-	-	-	-	-	-	-	-

Table 4: Percentages of major components (≥ 5%) of essential oil of M. spicata growing in Ouargla (Algeria) and other countries.

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DECLARATION OF INTEREST

There is no conflict of interest.

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