Chemical Composition, Antioxidant and *In vitro* Antibacterial Activities of Essential Oils of *Mentha spicata* Leaf from Tiaret Area (Algeria)

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ABSTRACT

The antioxidant and antibacterial activities and the composition of essential oil of *Mentha spicata* from Tiaret area were evaluated. The essential oil was obtained by hydrodistillation. The antioxidant activity was determined by DPPH radical scavenging assay and reducing power. The antimicrobial activity was evaluated by the broth micro-dilution method against seven strains isolated from diarrhea of young calves. The essential oil was analyzed by GC-MS/GC-FID. A total of 116 compounds amounting of 99.92% of the total component of the oil were identified. The major component of the oil was carvone (63.59%).The antioxidant activity by DPPH assay showed IC_{50} value of 21.1 ± 7.17 µg/ml, while the reducing power assay have resulted an EC_{50}value of 2.28 ± 0.68 µg/ml. The results showed that the essential oil exhibited an MIC against all tested bacteria at 2.37 mg/ml to 4.75 mg/ml. The essential oil of *M. spicata* showed strong antioxidant and weak to moderate antibacterial activities.

Key words: *Mentha spicata*, essential oil, antimicrobial activity, antioxidant activity, enteropathogens bacteria.

INTRODUCTION

In the livestock industry, antimicrobial agents are used in chemoprophylaxis, chemotherapy and as growth promoters, but the misuse or abuse of these agents has the potential to result in a serious increase in resistance amongst bacteria.[1] Additionally, antimicrobial resistance is an emerging and serious public health concern due to the compromised efficacy of antimicrobial agents used in the treatment of infectious diseases.[2]

Medicinal plants are the boon of nature to cure a number of ailments of human beings. In many parts of the world, medicinal plants are used against bacterial, viral and fungal infections.[3] In fact, medicinal plants are rich sources of bioactive secondary metabolites, including polyphenols and essential oils. The latters are especially obtained from aromatic plants by steam distillation or solvent extraction. Essential oil contains several chemical compounds exhibiting different biological activities. It can reduce foodborne pathogens and decrease the use of synthetic and semisynthetic antimicrobial compounds.[4]

The genus *Mentha* includes 25-30 species that grow under cultivation from tropical to temperate climate of many countries.[5] *Mentha spicata* is one the most common and popular mint cultivated throughout the world.[6] In Algeria, this species is locally known as 'Naanaa' and is cultivated all over

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the country for culinary purposes as well as for its medicinal properties.[7] The leaves are popularly used as tea flavouring agent and the whole plant is used as carminative. The fresh and dried plants and their essential oils are widely used in the food, cosmetic, confectionary, chewing gum, toothpaste, and pharmaceutical industries.[5] Likewise, *M. spicata* has been extensively applied to treat various diseases such as nausea, vomiting, and gastrointestinal disorders and also as breath freshener, antiseptic mouth rinse, and in toothpaste.[6]

The aim of the present study was to determine the chemical composition of the essential oil of Algerian spearmint, growing in the Dahmouni Locality (Tiaret), and to evaluate their antioxidant and antimicrobial effects against several pathogenic bacteria isolated from diarrhea of young calves.

MATERIALS AND METHODS

Extraction of essential oil. Aerial part of *M. spicata* was harvested in June, July and August 2014 in Ouled Bougadou (Dahmouni, Tiaret) located at west Algeria. The collected leaves were cut into small pieces and shade dried at room temperature and obscurity. The essential oil was extracted by hydro-distillation for 1H 30' by mixing 25g of *M. spicata* in 500 ml of distilled water. The oil obtained was collected and dried over anhydrous sodium sulfate and stored in sealed glass vials in a refrigerator at 4°C prior to analysis. The percentage yield of *M. spicata* essential oil has been calculated using the following formula.

$$\text{Yield of essential oil} = \frac{\text{Essential oil weight (g)}}{\text{Sample weight (g)}} \times 100$$

Analysis of essential oil. The essential oil from leaves of *M. spicata* was analysed by gas chromatography (GC-FID) and gas chromatography–mass spectrometry (GC/MS) realized by Pyrenessences Analysis. Briefly, the oil was analyzed using a Hewlett Packard 5973 instrument, with HP INNOWAX polar column (60 m × 0.25 mm × 0.25 µm). One microliter of essential oil solution diluted in ethanol (10%) was injected and analysed. Helium was the carrier gas with a flow rate of 30 psi/FID, 23 psi/MS. Column temperature was initially kept at 60°C for 6 min and then gradually increased to 250°C at 2°C/min rate and finally held for 10 min at 250°C. The temperature of the injector was fixed to 250°C and one of the detectors (FID) to 270°C. The compounds were identified by a combined search of retention time and mass spectra (NKS library, 75.000 spectra). The percentages were calculated from the peak areas given by the GC/ FID, without the use of correction factor.

Antimicrobial study

Microorganisms. The antimicrobial activity of the essential oil of *M. spicata* was evaluated against seven strains of Gram-negative bacteria (*Escherichia coli F5, E. coli F17, E. coli CS31A, Kluyvera* spp., *Klebsiella* spp., *Salmonella* spp. and *Serratia* spp.). The microbial strains used in this study were isolated from diarrhea of young calves.

Preparation of inoculum. Prior to the experiment, the strains were inoculated onto the surface of MacConkey agar media, the inoculum suspensions were obtained by taking five colonies from 24 h cultures. The colonies were suspended in 5 ml of sterile saline (0.85% NaCl) and shaken for 15 seconds. The density was adjusted to the turbidity of a 0.5 McFarland Standard (equivalent to 1 - 5 × 10^8 cfu/ml).

Minimum inhibitory concentration (MIC) measurement. The broth micro-dilution method employed for the determination of antimicrobial activities of the essential oils was performed according to the recommendations of the National Committee for Clinical Laboratory Standards.[8] Minimum inhibitory concentration determination was performed by a serial dilution method in 96 well microtiter plates. The starting concentration of the essential oil solution was 9.5 mg/ml. Further, stock solutions of the essential oils were prepared in 10% aqueous Tween 20 and then double serial dilutions of the oils were made. The inoculum was added to all wells and the plates were incubated at 37°C for 24 h. The bacterial growth was visualized by adding 20 µl

of 0.5% 2,3,5-triphenyltetrazolium chloride (TTC) aqueous solution.[9] Minimum inhibitory concentration (MIC) was defined as the lowest concentration of the oils that inhibited visible growth (red-colored pellet at the bottom of the wells after the addition of TTC), while the minimum bactericidal concentration (MBC) was defined as the lowest concentration that killed 99.9% of bacterial cells. To determine MBC, broth was taken from each well without visible growth and inoculated on Mueller Hinton agar for 24 h at 37^0C. The experiments were done in triplicate.

Antibiotic susceptibility test. Susceptibility to a panel of antimicrobial agents was determined by the standardized disc diffusion assay on Mueller-Hinton agar with commercial antimicrobial susceptibility discs according to the recommendations of committee on standardization of susceptibility testing in veterinary medicine.[10,11] The antibiotics tested and their corresponding disc concentrations were as follows: amoxicillin + clavulanic acid (20/10 µg), ampicillin (10 µg), gentamicin (10µg), tetracycline (10µg), colistin (10 µg), trimethoprime/ sulfa-methoxazole (1.25/23.75 µg), ofloxacine (5 µg) and cifotaxime (30 µg). The plates were then incubated at 37 °C for 24h to 48h. The diameter of zone of inhibition was recorded and the data was interpreted using the recommendation of committee on standardization of susceptibility testing in veterinary medicine.[10,11]

Antioxidant activity

Reducing power. The Fe^{3+} reducing power of essential oil was determined by the method of Yen and Duh[12] with some modifications. Essential oil was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated for 20 min at 50 °C. After incubation, 2.5 ml of trichloroacetic acid (10%) was added to the mixture followed by centrifugation at 3000 rpm for 10 min. The upper layer (1 ml) was mixed with 1 ml of distilled water and 0.5 ml of ferric chloride (0.1%). The absorbance of the obtained solution was measured at 700 nm. A higher absorbance indicates a higher reducing power. All tests were carried out in triplicate. Quercetin and ascorbic acid were used as positive controls.

DPPH radical-scavenging assay. The capacity to scavenge the "stable" free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was assessed according to the method described previously.[13] Two ml of various concentrations of essential oil of *M. spicata* was added to 0.4 ml solution of DPPH radical in ethanol (final concentration of DPPH was 0.5 mM). The mixture was shaken and kept in the dark for 30 min and the absorbance of the resulting solution was measured at 517 nm. Inhibition of free radical DPPH in percent (DPPH I%) was calculated as follows:

$$\text{DPPH I\%} = 100 \text{ X } (A_{blank} - A_{sample})/A_{blank},$$

where A_{blank} represents the absorbance of the control reaction (containing all reagents except the tested compound), and A_{sample} represents the absorbance of the tested compound. Gallic acid, quercetin and ascorbic acid were used as positive controls.

Statistical analysis. The assays were performed in triplicate and the results were expressed as the mean values with standard deviations (SD). The significant differences were obtained by a one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) post hoc test ($p < 0.05$). These statistical analyses were calculated using R software (version 3.3.0/ 2016-05-03).

RESULTS AND DISCUSSION

The yield of the oil obtained from *M. spicata* was 1.97 ± 0.09%. Our results are in agreement with those of Soković *et.al.*[14,15] They found that the yield of essential oil was 1.5 %. The yield of essential oils from *M. spicata* leaves harvested in three other regions of Algeria (Bejaia Saida and Setif) was 1.1%, 1.3% and 0.89%, respectively.[7,16,17]

However, the extraction yield of Tunisian spearmint was 1.1%,[5] Pakistan spearmint 1.2%,[18] Indian spearmint ranged from 0.4 - 0.8%,[19] and Bangladeshi spearmint was 0.33%.[20] On the other hand, Roldán *et al.*[21] and Martins *et al.*[22] have shown very lower rate with 0.08 and 0.1%, respectively.

However, *M. spicata* is known to have a minimum of 10 ml/kg of essential oil.[23]

The difference in yield can be attributed to the harvest season of the plant when a high essential oil yield was seen in summer (when the plants were in full bloom) than in winter (when the plants reached the end of their growing cycle).[7,24,25]

The composition of essential oils of *M. spicata* is presented in table 1. One hundred and sixteen compounds were identified accounting for 99.92% of the total amount. The main constituents were carvone (63.59%), followed by limonene (5.85%), dihydrocarvyl acetate (4.17%), 1,8-cineole + β-phellandrene (3.75%), trans-thujanol (2.74%), cis-dihydrocarvone (1.68%), β-caryophyllene (1.37%), terpinen-4-ol (1.23%), borneol (1.11%) and cis-carvyl acetate (1.07%).

There is large variation in the chemical composition of *M. spicata* between the wild as well as cultivated samples around the world. Indeed, a series of chemotypes have been described in previous studies. Recently, Brahmi *et al.*[7] studied the chemical composition of *M. spicata* essential oil originating from Bejaia locality (Algeria) and reported that carvone (48.5%), limonene (20.8%), 1,8-cineole (5.4%), β-caryophyllene (3.4%), germacrene D (3.4%) and β-pinene (1.1%) as the major components. Likewise, Allali *et al.*[16] reported carvone (48.42%), eucalyptol (17.6%) and neoisodehydrocarveol acetate (11.7%) as the main components of the essential oil collected from Saida (west northern region of Algeria). *M. spicata* collected from Amoucha, Setif locality (north east Algeria) showed carvone (59.40%) followed by limonene (6.12%), 1,8-cineole, germacrene-D (04.66%), β-caryophyllene (2.969 %), β-bourbonene (2.796 %), α-terpineol (1.986 %) and terpinen-4-ol (1.120 %) in its oils.[17] *M. spicata* growing in other countries also contains carvone as one of the major components of its essential oil, e.g., Portugal (41.1%)[22], Morocco (29.0%)[26], Bangladesh (73.29%)[20] and Serbia (49.52%).[15]

Nevertheless, Dhifi *et al.*[27] demonstrated an entirely different oil composition of *M. spicata* harvested in Tunisia, where menthone (32.7%) and pulegone (26.6%) was found as the major constituents. Even, Telic *et al.*[28] in Turkey, reported linalool (82.8%) chemotype of *M. spicata.* The variations in chemical composition of the essential oils with respect to season might have been due to the influence of phenological status, and environmental conditions can influence the regulation of the biosynthesis of essential oil. Previous investigations have demonstrated that harvesting season can alter the chemical composition of the essential oils of *M. spicata*.[24] Other explanation for the differences in oil content and composition may be attributed to factors related to ecotype, phenophases, temperature, relative humidity, photoperiod, irradiance, genotype, and agronomic conditions (harvesting time, plant age, crop density).[5] In addition, the duration of sunshine, the movement of air and precipitation can play roles in the difference in composition of essential oils.[17]

Two antioxidant assays have been used to evaluate the possible antioxidant properties of the spearmint oil, including ferric reducing power and DPPH radical scavenging activity. The results obtained (Table 2) confirm the previous reports about the importance of essential oils as natural antioxidants. Reducing power measures the ability of an antioxidant to donate an electron.[29] These antioxidants trap the free radicals, act on certain peroxide precursors, and prevent the peroxidation chain reaction.[30] The assayed sample was able to reduce the ferric ions (Fe^{3+}) to corresponding ferrous ions (Fe^{2+}). The EC_{50} value for spearmint essential oil was 2.28 ± 0.68 µg/ml in comparison to 21.22 ± 1.66 µg/ml and 13.98 ± 1.99 µg/ml for the standard compound quercetin and ascorbic acid, respectively. Brahmi *et al.*[7] and Snouci *et al.*[5] reported that the EC_{50} value of the *M. spicata* essential oil harvested from north Algeria and northeast of Tunisia were about 452.3 ± 0.4 µg/ml and 2.49 ± 0.07 µg/ml, respectively.

Table 1. Chemical composition of essential oil.

Pick	RT (min)	Components	%
1	7.2	Ethanol	0.1
2	10.5	α-Pinene	0.48
3	10.6	α-Thujene	0.08
4	11.4	Trans-2,5-diethyltetrahydrofuran	0.01
5	11.7	Ethyl 2-methylbutyrate	0.01
6	12.6	Camphene	0.2
7	14.7	β-Pinene	0.74
8	15.4	Sabinene	0.56
9	17.6	β-Myrcene	0.61
10	18.0	α-Phellandrene	0.01
11	18.2	ψ-Limonene	0.02
12	18.9	α-Terpinene	0.17
13	20.1	Limonene	5.85
14	20.9	1,8-cinaeole + β-phellandrene	3.75
15	21.2	1,3,8-p-menthatriene	0.03
16	21.4	2-hexenal	0.02
17	22.1	Cis-β-ocimene	0.18
18	23.1	γ-terpinene	0.34
19	23.3	Trans-β-ocimene	0.05
20	24.8	*p*-cymene	0.23
21	25.1	2-Metthyl butyl 2- methyl butyrate	0.01
22	25.3	3-Heptanol	0.02
23	25.6	Terpinolene	0.1
24	28.9	1-Ethylhexyle acetate	0.12
25	30.5	Aliphatic ester	0.01
26	32.6	3-Octanol	0.4
27	33.3	Nonanal	0.03
28	35.9	Aliphatic ester	0.02
29	36.2	α, p-Dimethylstyrene	0.02
30	36.4	1-Octen-3-ol	0.06
31	37	Cis-1,2-limonene epoxide	0.01
32	37.8	Trans-thujanol	2.74
33	38	Trans-limonene 1,2- epoxide	0.06
34	38.4	Cis-2- hexenyl methyl butyrate	0.01
35	38.5	Menthone	0.01
36	39.3	3-Nonanol	0.01
37	39.4	Cis-3-hexenyl methyl butyrate	0.08
38	40.2	α-Copaene	0.02
39	40.3	Isomenthone	0.02
40	41.6	α-Bourbonene	0.03
41	42	β-Bourbonen	0.41
42	42.2	Dihdroedulan isomer	0.03
43	42.9	Linalool	0.03

44	43.1	Aliphatic alcohol	0.03
45	43.4	Cis-thujanol	0.21
46	43.6	1-Octanol	0.02
47	44.1	Isomeric limonene epoxide	0.01
48	44.4	Trans-p-menth-2-en-1-ol	0.1
49	45.7	ε-Cadinene	0.08
50	45.9	Bornyl acetate	0.09
51	46.5	β-Elemene	0.09
52	46.9	β-Cubebene	0.06
53	47.1	Terpinen-4-ol	1.23
54	47.3	β-Caryophyllene	1.31
55	48	Cis-dihydrocarvone	1.68
56	48.5	Cis-p-2,8-menthadien-1-ol	0.06
57	49.1	Trans-dihydrocarvone	0.15
58	49.8	Cadina-3,5-	0.1
59	50	Isomeric cadinadiene	0.05
60	50.2	Allo-aromadendrene	0.03
61	50.4	Trans-pinocarveol	0.02
62	50.9	E-β-Farnesene	0.04
63	51.2	Trans-p-2,8-menthadien-1-ol	0.05
64	51.3	δ-Terpineol	0.23
65	51.5	Dihydrocarvyl acetate	4.17
66	51.9	α-Humulene	0.14
67	52	Cis-4,5-Muuroladiene	0.47
68	52.3	Sesquiterpene	0.06
69	52.9	α-Terpineol	0.32
70	53.2	Borneol	1.11
71	54.4	Germacrene D	0.96
72	54.7	Dihydrocarveol isomer	0.16
73	55.4	Trans-carvyl acetate	0.03
74	56.1	Carvone	63.59
75	56.3	Bicyclogermacrene	0.4
76	56.9	δ-Cadinene	0.04
77	57.2	γ-Cadinene	0.07
78	57.5	Cis-carvyl acetate	1.07
79	58.1	Methyl salicylate	0.03
80	58.8	Nerol	0.02
81	58.9	Perillaldehyde	0.03
82	59.2	α-amorphene	0.09
83	59.3	Terpenic alcohol	0.04
84	59.9	Terpenic ester	0.02
85	60.9	Trans-carveol	0.21
86	61.5	Calamenene	0.35
87	61.6	p-cymen-8-ol	0.04
88	62	Carvone oxide	0.03
89	62.3	E-geranyl acetone	0.03
90	62.7	Cis-carveol	0.58

91	66.8	Piperitenone		0.2
92	67.7	Trans-jasmone		0.66
93	68.5	Piperitenone oxide		0.03
94	69	2-phenylethyle 2-methylbutyrate		0.02
95	69.9	Aliphatic ester + aromatic compound		0.05
96	70.4	Caryophyllen oxide		0.16
97	71.1	Sesquiterpenol		0.02
98	72.2	Sesquiterpenol		0.01
99	72.3	Sesquiterpenol		0.02
100	73.3	Germacra-1,5-dien-4-ol		0.05
101	73.9	Epi-cubenol		0.37
102	75.2	Methyl p-anisate		0.03
103	77.1	Spathulenol		0.17
104	78.8	Eugenol		0.08
105	79.4	T-Cadinol		0.07
106	80.2	α-Muurolol		0.03
107	80.7	Carvacol		0.03
108	81.5	α-Bisabolol		0.03
109	82.1	Isospathulenol isomer		0.03
110	82.4	α-Cadinol		0.27
111	84.7	Sesquiterpenol		0.07
112	85	Aromatic compound		0.02
113	85.6	Caryophylla-3,7-dien-6-ol		0.02
114	86.3	Aromatic compound		0.04
115	88.4	Sesquiterpenic epoxide		0.02
116	88.7	Sesquiterpenic epoxide		0.03
			Total	99.92

DPPH assay has been widely used for the evaluation of free radical scavenging activity of plant extracts, food material or single compounds.[31] It has been established that the bleaching of the DPPH solution increased regularly with increasing amount of polyphenols.[29] Table 2 summarizes the results of the antiradical activity expressed as percentage inhibition of DPPH. The IC_{50} value for *M. spicata* essential oil was 21.19 ± 7.17µg/mL in comparison to the standard compound.

The IC_{50} obtained in our study is superior to those cited by Snoussi *et al.*[5] with a value of 3.08 ± 0.07µg / ml, Hussain *et al.*[18] showed the IC_{50}value of 13.3 ± 0.6 µg/ml, and Dhifi *et al.*[27] (10 ± 0.24 µg / ml) but much lower than those registered by Brahmi *et al.*[7], Moldovan *et al.*[32], Allali *et al.*[16], Soni and Sosa[33], Ebrahimzadeh *et al.*[34] and Nickavar *et. al.*[35] with 9544.6 ± 0.196 µg/ml, 151.05 ± 1.95 µg/ml, 10620 µg/ml, 170 µg/ml, 105.8 ± 3.98 µg/ml and 87.89 µg/ml, respectively. Snoussi *et al.*[5] and Soni and Sosa[33] showed that the essential oil IC_{50} was lower than standards used, which is in agreement with our study. This interesting biological activity can be explained by the presence of the monoterpenes limonene, terpinolene, γ-terpinene, 1,8-cineole and carvone.[5]

Regarding the antibiotic susceptibility, all tested strains were resistant to ampicillin, amoxicillin + clavulanic acid, tetracycline, trimethoprime/ sulfamethoxazole and ofloxacine (table 3). The antimicrobial activity of essential oil of *M. spicata*a gainst Gram-negative bacteria associated with neonatal calves diarrhoea is shown in table 4.

In our study, the essential oil of *M. spicata* showed moderate activity against bacterial strains

selected. Results obtained from minimum inhibitory concentration method, indicated that *E. coli* F5, *E. coli* CS31A, *Salmonella* spp., *Kluyvera* spp. and *Klebsiella* spp. were the most sensitive bacteria among microorganisms tested, with lowest MIC values (2.37 mg/ml). The lowest activity was observed for *E. coli* F17 with highest MIC value (>9.5 mg/mL). The value of MBC 9.5 mg / ml were sufficient to kill *E. coli* CS31A and *Klebsiella* spp., whereas higher values of MBC (> 9.5 mg/ml) are needed to kill others bacteria tested in this study.

Table 2. Antioxidant activity of essential oil of *M. spicata* in reducing power and DPPH assays.

	Reducing power (EC_{50}) µg/ml	DPPH (IC_{50}) µg/ml
Quercetin	21.22 ± 1.66^{c}	$12.02 \pm 1.3^{a,b}$
Gallic acid	-	5.39 ± 0.41^{b}
Ascorbic acid	13.98 ± 1.99^{b}	6.19 ± 0.97^{b}
Essential oil of *M. spicata*	2.28 ± 0.68^{a}	21.19 ± 7.17^{a}

Each value in the table is represented as mean ± SD (n = 3). Means not sharing the same letter are significantly different (LSD) at P <0.05 probability level in each column

Table 3. Antimicrobial susceptibility of selected microbial strains.

Antibiotic	A	B	C	D	E	F	G
Ampicillin (10 µg)	R	R	R	R	R	R	R
Amoxicillin+ clavulanicacid (20/10 µg)	R	R	R	R	R	R	R
Gentamicin (10 µg)	R	R	R	R	S	I	S
Tetracycline (10 µg)	R	R	R	R	R	R	R
Colistin (10 µg),	S	R	R	S	S	S	R
Trimethoprime/sulfamethoxazole (1.25/23.75 µg)	R	R	R	R	R	R	R
Ofloxacine (5 µg)	R	R	R	R	R	R	R
Cifotaxime (30 µg)	S	S	R	S	S	I	R

A: *E. coli* F5, *B: E. coli* F17, *C: E. coli* CS31A, *D: Klebsilla* spp., *E: Kluyvera* spp., *F: Salmonella* spp., *G: Serratia* spp.

Table 4. Antimicrobial activity of essential oils of *M. spicata.*

Bacteria	Essential oil of *M. spicata* (mg/ml)	
	MIC	MBC
Serratia spp.	4.75	> 9.5
Salmonella spp.	2.37	> 9.5
Kluyvera spp.	2.37	> 9.5
Klebsiella spp.	2.37	9.5
E. coli F5	2.37	> 9.5
E. coli F17	> 9.5	> 9.5
E. coli CS31A	2.37	9.5

Many previous studies reported a small MIC ranging from 0.21 ± 0.01mg/ml, 0.345 ± 0.01mg/ml to 0.349 ± 0.008 mg/ml against *E. coli*.[18,24] Likewise, Martins *et al*.[22] showed the MIC values ringing from 250 µg/ml to 500 µg/ml against *E. coli*, *Salmonella enteritidis* and *Klebsiella pneumonia.* The lowest value of MIC < 3.5µg/ml was reported by Sarer *et al*.[36]

M. spicata essential oil was detected as active against only *Klebsiella* spp. and *E. coli CS31A* with MBC of 9.5 mg/ml. Similar results were reported by Roldan *et al*.[21] against *E. coli ATCC 25922, E. coli O157:H7, Salmonella enteritidis* ATCC13076 and *Salmonella typhimurium* ATCC14028. However, Sokovic *et al*.[14] signalled lowest result of MBC (3 µg/ml). The antibacterial activity of *M. spicata* essential oil could be attributed to the presence of carvone and limonene. It has been reported that carvone is one of the most efficient antimicrobial agents of various plants. The mechanism of antibacterial activity of carvone is not completely understood in great detail. It has been demonstrated that the mechanism of action of carvone on the growth of microorganisms includes the destabilization of the phospholipid bilayer structure, interaction with membrane enzymes and proteins,[6] it act as a proton exchanger reducing the pH gradient across the membrane[6,37] and probably it could act by disrupting the metabolic energy state of the cells. In contrast, another study found that carvone was ineffective against the outer membrane of *E. coli* and *S. typhimurium* and did not affect their intracellular ATP pool.[37]

The present study revealed that the main component of the essential oil of *M. spicata* cultivated in the Tiaret region was the carvone, which is well corroborated with the results obtained in other Algerian regions. The essential oil of *M. spicata* showed a strong antioxidant activity and a weak to moderate antibacterial activity. These bioactivities are mainly due to the high content of carvone, in this species.

Authors contributions

Selles SMA supervised, controlled the laboratory work and wrote the first draft. Ait Amrane Ammar and Kouidri M designed the manuscript. Belhamiti TB and Benia AR helped to analyse experimental results. Bellik Y helped in data interpretation and discussion. Hammoudi SM and Boukraa lrevised the manuscript. All authors read and approved the final manuscript.

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