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Chemical and biological profiles of essential oils from *Mentha spicata* L. leaf from Bejaia in Algeria

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ABSTRACT

Air-dried leaves of *Mentha spicata* L. were collected in Bejaia location (Algeria) during 2013. The plant samples were distillated by Clevenger apparatus and the essential oils obtained were analyzed by GC and GC/MS. Fifty compounds amounting to 98.9% of the oil, were identified. The major components of the oil were carvone (48.5%), limonene (20.7%), and 1,8-cineole (5.4%). This oil exhibited high antimicrobial activity with a high effectiveness against *Candida albicans* strain with a diameter of growth inhibition zones of ranging from 14.3 ± 1.5 to 44.3 ± 1.1 mm. The antioxidant activity determined by five different test systems showed that the oil exhibits moderate activity. The insecticidal activity has been tested on the adults of *Rhyzopertha dominica* F., the main pest of wheat. Contact and fumigant toxicity assays showed mortality percentages of 14.0 and 34.5% respectively at the dose of 2 µL/mL and the repellency percentage was 56.2% at 30 minutes.

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Mentha spicata L; essential oil; antimicrobial activity; insecticidal activity; antioxidant activity

Introduction

The genus *Mentha* L. (Lamiaceae), comprising more than twenty-five species, is responsible for approximately 2000 t of world essential oil, making it the second most important essential oil-producing genus, after *Citrus* (1). *Mentha spicata* L., a member of this genus, commonly known as spearmint, is a long-used medicinal herb found in many African countries including Algeria. *M. spicata* (synonymous with *M. viridis Linn.*) is an herbaceous perennial with a pungent smell. It is commonly used as an herbal tea, flavoring agent, and as a medicinal plant (2).

The herb is considered to have stimulant, carminative, antispasmodic, stomachic and diuretic properties. It is also used for gas pain, rheumatism, toothache, muscle pain and as a mouth wash (3). *M. spicata* L., locally known as 'Naanaa', is cultivated all over Algeria for culinary purposes as well as for its medicinal properties particularly to treat gastric troubles (2).

Spearmint's distinctive flavor and strong, warm aroma is due to its essential oil content. There is an ongoing effort to screen plants used medicinally in different regions of the world. However, it is well known that the same taxon growing in different areas may have widely differing chemical components and hence differing biological properties (4, 5).

Literature mentions a few studies that have been done that either measure only antioxidant and antibacterial properties of the *Mentha spicata* L. (6), or only anti-fungal, anti-aflatoxigentic, and insecticidal properties. These studies were conducted on plants found outside Algeria (7). This study examines, antioxidant, antimicrobial and insecticidal activities of the essential oils of *Mentha spicata* L, grown specifically in Algeria and collected from the Bejaia.

The aim of this work was to study the chemical composition of the essential oils of Algerian spearmint, grown in the Kabily mountains (Bejaia), and to evaluate their insecticidal, antioxidant, and antimicrobial effects against several pathogenic microorganisms.

Experimental

Plant material and extraction of essential oil

The plant material was collected before flowering in March 2013 from Algerian Bejaia locality (181 km in the east of Algiers): latitude: 5°2'8.88"E longitude: 36°45'1.49"N; altitude:

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32 m). The climate is semi-humid with an annual average temperature of 17.6°C. It was authenticated by Professor Lejoly (Laboratory of Systematical Botany and Phytosociology, Free University of Brussels (ULB), Belgium) and a voucher specimen was deposited in the Herbarium of the National Botanical Garden of Meise (Belgium) under the number BR 0000006946227. The leaves of *M. spicata* were shade dried at room temperature with ventilation and hydro-distilled (100 g) for 3 hours using a Clevenger-type apparatus. 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. Yield was calculated based on the dried weight of the sample.

Analyses of the essential oil

Analyses of the essential oils were carried out by gas chromatography (GC) and by gas chromatography–mass spectrometry (GC–MS). GC–MS analyses were carried out on a gas chromatograph (Agilent, Model 6890N, Palo Alto, CA) equipped with a split–splitless injector, an autosampler Agilent model 7683, and Agilent fused silica capillary columns (30 m × 0.25 mm i.d., film thickness 0.25 μm) (HP-5, 5% phenyl-methylpolysiloxane). Column temperature was programmed heating from 60 to 250°C at 3°C/minute followed by 20 minutes under isothermal conditions. The injector was maintained at 250°C. Helium was the carrier gas at 1.0 mL/minute and the sample (1 μL) was injected in the split mode (1:10). The GC was fitted with a quadrupole mass spectrometer, MS, Agilent model 5973 detector. MS conditions were as follows: ionization energy 70 eV, electronic impact ion source temperature 200°C, quadrupole temperature 150°C, scan rate 3.2 scan/s, mass range 30–480 u. ChemStation software was used to handle mass spectra and chromatograms. Samples were run in hexane with a dilution ratio of 1:100. Compounds were identified by comparison of their mass spectra with those of the NIST02 library data of the GC–MS system and Adams library spectra (8, 9). The results were further confirmed by comparison with the compounds elution order with their retention indices on semi-polar phases reported in the literature (9). Retention indices of the components were determined relative to the retention times of a series of n-alkanes (2 standard mix C8–C20 and C21–C40) (Sigma) with linear interpolation (10). Percentage of individual components was calculated based on GC peak areas without FID response factor correction.

Antioxidant activity

1, 1-Diphenyl-2-picrylhydrazyl (DPPH) and 2, 2-Azino Bis-3-ethylbenzoThiazoline-6-Sulphonate radical cation ($ABTS^{\bullet+}$) activities

The effect of the tested essential oil on DPPH degradation was estimated according to the method described in the literature (11). The essential oil was diluted in pure ethanol at different concentrations, and then 2 mL were added to 0.15 mL of a 10^{-3} mmol/L DPPH ethanolic solution. The mixture was shaken vigorously and left standing at room temperature for 60 minutes. The absorbance of the resulting solution was then measured at 517 nm. The antiradical activity (three replicates per treatment) was expressed as IC_{50} (μg/mL), the antiradical dose required to cause a 50% inhibition. A lower IC_{50} value corresponds to a higher antioxidant activity of essential oil. The ability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = [(A_0 - A_1) \times 100]/A_0$$

Where A_0 is the absorbance of the control at 60 minutes, and A_1 is the absorbance of the sample at 60 minutes.

The radical scavenging capacity of the radical cation ($ABTS^{\bullet+}$) was determined by the reported method (12). $ABTS^{\bullet+}$ was generated by mixing a 7 mM of ABTS with 2.45 mM potassium persulfate followed by storage in the dark at room temperature for 16 hours before use. The mixture was diluted with ethanol to give an absorbance of 0.70 ± 0.02 units at 734 nm using a spectrophotometer. The essential oil (10 μL) was allowed to react with fresh $ABTS^{\bullet+}$ solution (990 μL), and then the absorbance was measured 30 minutes after initial mixing. The capacity of free radical scavenging was expressed by IC_{50s} (μg/mL) values, which represents the concentration required to scavenge 50% of $ABTS^{\bullet+}$ radicals. The free radical scavenging IC_{50} capacity was determined using the same equation previously used in the DPPH method. Radical scavenging capacity of the tested oil was compared to that of butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), α-tocopherol and ascorbic acid.

Reducing power (RP) and total antioxidant activity (TAA) by phosphomolybdenum method

The ability of the extracts to reduce Fe^{3+} was assayed by the method of Oyaizu (13). Briefly, 1 mL of *M. spicata* essential oil was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% $K_3Fe(CN)_6$. After incubation at 50°C for 25 minutes, 2.5 mL of 10% trichloroacetic acid was added and the mixture was centrifuged at 650g for 10 minutes. Finally, 2.5 mL of the upper layer was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% aqueous $FeCl_3$. The absorbance was measured at 700 nm. The mean of absorbance values were plotted against concentration and a linear regression analysis was carried out. RP_{50} value (μg/mL) is the effective concentration at which the absorbance was 0.5 for reducing power.

TAA was measured as per the method reported by Brahmi et al. (2). One milliliter of reagent solution containing 0.6 M H_2SO_4, 28 mmol/L sodium phosphate and 4 mmol/L ammonium molybdate was added to different concentrations of the sample. The samples were then heated at 95°C for 90 minutes in a hot water bath. The mixture was then cooled and the absorbance was read at 695 nm. The TAA values have been expressed as RP_{50} (µg/mL) which is the effective concentration at which the absorbance was 0.5 and was obtained by interpolation from linear regression analysis. Ascorbic acid, α-tocopherol, BHT and BHA were assayed for comparison.

Determination of antioxidant activity by using the KRL biological test

The antioxidant defenses were further examined using a test based on *in vitro* free radical-induced blood hemolysis with the Kit Radical Libres (KRL) biological test. The blood solutions were diluted in phosphate buffer in isotonic conditions at pH = 7.4. The essential oils were dissolved in DMSO at different concentrations. Blood solutions were incubated at 37°C with different range of concentrations of the essential oils for 15 minutes before being submitted to free radicals produced by a final 50 mM solution of 2, 2'-azobis (2-amidinopropane) hydrochloride (AAPH). Hemolysis was recorded using a 96-well microplate reader by monitoring absorbance at 620 nm (Laboratoires Spiral, France). Results, given in triplicate, were expressed as 50% of maximal hemolysis time (half-hemolysis time, HT50 in minutes) and standardized in Trolox® and gallic acid equivalents (14).

Antimicrobial activity

The test microorganisms included the following Gram-positive bacteria: Methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 43300, *Staphylococcus aureus* NCCB 9163 and *Bacillus subtilis* ATCC 6633. The Gram negative bacteria were: *Escherichia coli* ATCC 25922, *Pseudomonas aeroginosa* ATCC 27853 and *Klebsiella pneumoniae* E47. The antimicrobial effect was also tested against two *Aspergillus* species (*niger* 2CA 936 and *flavus* NRRL 391) and *Candida albicans* ATCC 1024 strain.

For the experiments, the microorganisms working stocks were enriched and then incubated at 37°C for 18–24 hours for bacteria and at 25°C for 7 days for fungi. These cultures were the ones used to test the antimicrobial activity of essential oils and the optical density was adjusted at 0.5 McFarland turbidity standards. The inoculums of the respective bacteria and fungus were streaked onto Mueller-Hinton or Potato Dextrose agar plates.

For the disc-diffusion method, sterile filter discs (diameter 6 mm) were impregnated with 5 µL of essential oils and placed on the appropriate agar mediums. In the spots method, the same volume of oil was deposited directly on the same mediums. Streptomycin (10 µg/disc) and penicillin (10 µg/disc) were used as positive reference standards in the method of disc-diffusion. After incubation at 37°C for 18–24 hours, the diameter of the inhibition zone was measured.

Each experiment was carried out in triplicate and the mean diameter of the inhibition zone was recorded.

Insecticidal activity

The pure culture of the lesser grain borer, *Rhyzopertha dominica* (F.) was obtained from the private farm Benkellat (AKBOU, Bejaia) in wheat stocks. Subcultures were maintained in plastic pots with the food medium. The pots were kept in an incubator at 33 ± 1°C without light or humidity control. Wheat grains (*Triticum durum*) were used as the food medium throughout the experiment.

Contact toxicity

Contact toxicity was tested using groups of twenty adult insects to evaluate the mortality effect. An appropriate quantity of spearmint oil was diluted in acetone to obtain each test solution. A 500 µL aliquot of test solution containing 0.5, 1, 1.5, 2 and 0 (control) µL/mL was poured onto a Petri dish (9 cm diameter) containing 10 g of sterilized wheat grains. The treated Petri dishes were kept in an incubator at 33 ± 1°C. Mortality rate was checked daily for 4 days (96 hours). Four replicates were set up for each spearmint oil concentration and control. The insecticidal activity of the oil was expressed as % mean mortality.

These data were subjected to probit analyses using XLSTAT, Pro (2013) for Windows to estimate the Lethal Dose (LD_{50}) value of the essential oils.

Fumigation bioassay

In order to test the toxicity of essential oils on the adults of *Rhyzopertha dominica* (F.), 20 adults were put into the 500 mL glass jars. Essential oils were applied to a bit of cotton that was attached to the lower side of the jar's lid. The doses of spearmint oil used were 0.5, 1, 1.5, 2 and 0 (control) µL/mL. The glass jars were kept

at 33 ± 1°C. Mortality counts were made at 24, 48, 72 and 96 hours after treatment. Four replications were used for each dose.

Repellency test on filter paper

Essential oils of *M. spicata* were tested for repellence adapting a method suggested by Conti et al. (15). The method was adapted as follows: half filter paper discs (9 cm diameter) were treated with 0.5 mL of essential oil solutions in acetone at doses 0.5, 1, 1.5 and 2 µL oil per mL and the filters were air dried for 5 minutes. Half the bottom of a Petri dish (9 cm diameter) was covered with treated filter paper, while the other half was covered with a half filter paper disc treated with 0.5 mL of acetone only. Twenty unsexed adults were introduced to the middle of each Petri dish, and the lid was sealed with Parafilm®. Four replicates were run for each of the tested concentrations, so that 80 adults per concentration were assayed. Observations were taken after 30 minutes after the testing began: in each of them, the number of insects on the two half paper discs was recorded. Percentage repellency (PR) values were calculated as follows:

$$PR(\%) = \frac{(NC - NT)}{(NC + NT)} \times 100$$

NC: the number of insects on the half filter paper disc treated with 0.5 mL of acetone

NT: the number of insects on the half filter paper treated with 0.5 mL of essential oil solutions in acetone.

The mean repellency value was calculated and assigned to repellency classes from 0 to V according to Juliana and Su (16) scale: class 0 (PR\0.1%), class I (PR = 0.1–20%), class II (PR = 20.1–40%), class III (PR = 40.1–60%), class IV (PR = 60.1–80%) and class V (PR = 80.1–100%).

Statistical analysis

Relative percentage values in the contact and fumigant toxicity tests were corrected using the Abbott's formula (17):

$$MC(\%) = \frac{(M - Mt)}{(100 - Mt)} \times 100$$

Where MC is the corrected insect mortality, M is the insect mortality in the treated population of insect and Mt is the insect mortality in the control.

All experiments were conducted in triplicates and results are expressed as mean ± standard deviation (SD). Data were subjected to analysis of variance, and means were compared by least significant difference (LSD). Differences at $P < 0.05$ were considered to be significant.

Results and discussion

Essential oil composition

The oil yield of *M. spicata* was 1.1%. Our results are in agreement with those of Kofidis et al. (18). These authors found that the yield of essential oil from wild *M. spicata,* grown in Greece ranged from 0.1 to 1.8%. However, the extraction yield of Bangladesh spearmint was 0.4% (19) and in India was 0.3% (20). The yield found by Boukhbti et al. (21) from leaves of *M. spicata* 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% (22).

According to Hussain et al. (23) *Mentha* species demonstrated the highest essential oil yield in summer, when the plants were at full bloom, as opposed to in winter, when the plants reached the end of their growing cycle. The *M. spicata* provided 1.2% essential oil yields when harvested in summer and 0.95% when harvested in winter.

The gas chromatogram of the oil on a HP-5 MS capillary column is shown in Figure 1. The GC–MS analysis of the essential oil of *M. spicata* L. resulted in the detection of fifty components representing 98.9% of the oil (Table 1). The essential oil was found to be rich in oxygenated monoterpenes (60.1%) and monoterpene hydrocarbons (26.6%). The major components of the oil were carvone (48.5%), limonene (20.7%), 1,8-cineole (5.36%), β-caryophyllene (3.4%), and germacrene D (3.4%) as indicated in Figure 1.

A survey of the literature already published on this subject (5, 6, 21–25) reveals that several studies have already been reported on *M. spicata* essential oils composition, yet there are no reports on the composition of *M. spicata* specifically from the Bejaia location.

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 (18). *M. spicata* from Bejaia location (Algeria) was characterized by high percentage of carvone. The chemical composition revealed that the leaves had composition similar to those of other *M. spicata* essential oils analyzed from China by Zhao et al. (25) and in Algeria (Setif), by Boukhebti et al. (21), who reported the major component was carvone. According to Zhao et al. (25), *M. spicata* accessions from different locations of China

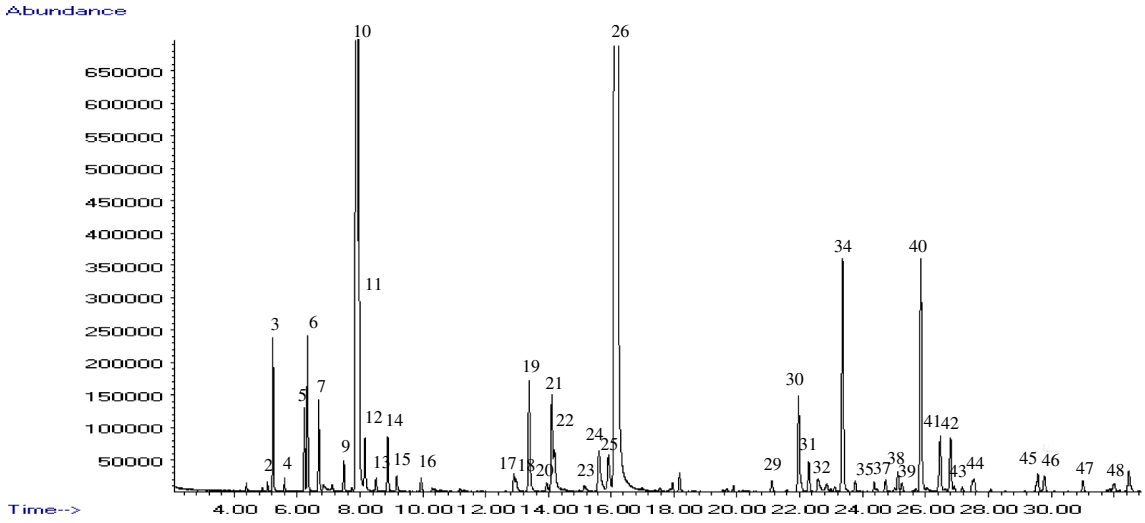

Figure 1. Chromatographic profile of *M. spicata* L. essential oil from Algeria. Compounds are numbered as listed in Table 1.

were relatively stable in essential oils content and composition and were grouped together into chemotype carvone. *M. spicata* produces almost exclusively monoterpenes bearing an oxygen function at C6 such as carvone (4).

Nonetheless, pulegone and piperitone were the major terpenoid group in the essential oil of Turkey spearmint (5). In fact, such divergence in the composition of the essential oil of *M. spicata* may be due to both developmental and environmental factors as well as to a difference in the levels of biosynthetic enzymes (4).

Antioxidant activity

Antioxidant activity of the essential oil of *M. spicata* has been determined by five different test systems, namely DPPH, ABTS, phosphomolybdate, reducing power (RP) (Table 2) and KRL biological test (Table 3). The assessed sample was able to reduce the stable violet DPPH$^{\bullet}$ radical to the yellow DPPH-H, reaching 50% of reduction with IC_{50} value of 9544.6 ± 196.2 µg mL. Although DPPH and ABTS were methods based on the same principle, data obtained from the ABTS assay were lower, but comparable, to those obtained from DPPH assay, reaching an IC_{50} value of 36.2 ± 3.2 µg/mL.

This is probably due to the steric factors which are major contributors to the reduction of the stable DPPH$^{\bullet}$ radical. While comparing our present results of this assay with the literature already published, we found very few reports on the radical scavenging activity of essential oils from *M. spicata*. Mkaddem et al. (24) found that the *M. spicata* essential oil was more effective to scavenge the ABTS$^{\bullet+}$ radical cation with an IC_{50} value of 195.1 ± 4.2 µg/mL than a DPPH$^{\bullet}$ (IC_{50} = 3476.3 µg/mL). Kizil et al. (26) and Hussain (23) reported that *M. spicata* essential oils notably reduced the concentration of the DPPH$^{\bullet}$ free radical, with an IC_{50} of 77.4 ± 1.1 and 12.7–18.5 µg/ mL, respectively. The IC_{50} obtained by Allali et al. (22) was 10620 µg/mL.

In order to compare the results given above, we tested the reducing ability of *M. spicata* essential oil using a spectrophotometric method. We used this method to measure the content of ferric ion and transition metal ion Mo (VI) which were reduced by the tested oil. The assayed sample was able to reduce the ferric ions (Fe^{3+}) to corresponding ferrous ions (Fe^{2+}), reaching a 50% reduction with an RP_{50} value of 452.3 ± 0.4 µg/mL. The corresponding oil was more effective in reducing Mo (VI) to Mo (V) with an RP_{50} value of 53.3 ± 2.8 µg/mL.

Results reveal that oil is much less effective when compared with synthetic antioxidant agents, BHT, BHA, α-tocopherol, and ascorbic acid.

The total antioxidant activity of *M. spicata* essential oils was also evaluated using the KRL biological test. The KRL test allows the evaluation of red blood cell resistance against the free radicals induced by 2, 2'-azobis (2-amidinopropane) hydrochloride (AAPH) that acts by producing peroxyl radicals, which induce lipid and protein peroxidation in the cell membrane (14).

Our results underline that *M. spicata* essential oils have also in this assay an antioxidant capacity, which does not increase linearly with the dose of the oil (Table 3). The best results were obtained at the concentration of 50 µg/mL. This means that the oil at this concentration has the ability to protect blood cells of oxidative stress. These essential oils should therefore be able to cross the plasma

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

N.	I_R [a]	$I_{R(litt)}$ [b]	Tr (min)	Compound	% Area	Identification[c]
1	897		4.4	n.i.	0.1	
2	925	924	5.1	α-Thujene	0.1	MS, I_R
3	932	932	5.2	α-Pinene	1.1	MS, I_R
4	947	946	5.6	Camphene	0.1	MS, I_R
5	972	969	6.2	Sabinene	0.7	MS, I_R
6	976	974	6.3	β-Pinene	1.3	MS, I_R
7	990	988	6.7	Myrcene	0.8	MS, I_R
8	995	988	6.8	3-Octanol	0.0	MS, I_R
9	1016	1014	7.5	α-Terpinene	0.3	MS, I_R
10	1028	1024	7.9	Limonene	20.8	MS, I_R
11	1029	1026	8.0	1,8-Cineole	5.4	MS, I_R
12	1035	1032	8.2	Z- β -Ocimene	0.6	MS, I_R
13	1046	1044	8.5	E- β -Ocimene	0.1	MS, I_R
14	1057	1054	8.9	γ-Terpinene	0.6	MS, I_R
15	1065	1065	9.2	*cis*-Sabinene hydrate	0.2	MS, I_R
16	1087	1086	9.9	Terpinolene	0.2	MS, I_R
17	1163	1165	12.9	Borneole	0.2	MS, I_R
18	1165	1166	13.0	p-Mentha-1,5-dien-8-ol	0.2	MS, I_R
19	1175	1174	13.4	Terpinen-4-ol	1.5	MS, I_R
20	1189	1186	13.9	α-Terpineol	0.1	MS, I_R
21	1193	1196	14.1	Neodihydrocarveol	1.3	MS, I_R
22	1196	1191	14.2	*cis*-Dihydro carvone	0.6	MS, I_R
23	1219	1215	15.1	trans-Carveol	0.1	MS, I_R
24	1230	1226	15.6	*cis*-Carveol	0.9	MS, I_R
25	1238	1233	15.9	Pulegone	0.6	MS, I_R
26	1244	1239	16.2	Carvone	48.5	MS, I_R
27	1286		18.0	n.i.	0.1	
28	1292		18.2	n.i.	0.2	
29	1362	1365	21.1	*cis*-Carvyl acetate	0.2	MS, I_R
30	1383	1387	22.0	β-Bourbonene	1.4	MS, I_R
31	1391	1389	22.3	β-Elemene	0.4	MS, I_R
32	1397		22.6	Z-Jasmone	0.3	MS, I_R
33	1404		22.9	n.i.	0.2	
34	1417	1417	23.4	β-Caryophyllene	3.4	MS, I_R
35	1427	1430	23.8	β-Copaene	0.1	MS, I_R
36	1442		24.4	n.i.	0.1	
37	1451	1452	24.7	α-Humulene	0.1	MS, I_R
38	1461	1461	25.1	*cis*-Cadina-1(6),4diene	0.3	MS, I_R
39	1464	1465	25.3	*cis*-Muurola-4(15),5-diene	0.1	MS, I_R
40	1479	1484	25.9	Germacrene D	3.4	MS, I_R
41	1494	1500	26.5	Bicyclogermacrene	0.8	MS, I_R
42	1502	1505	26.8	Premnaspirodiene	0.7	MS, I_R
43	1520	1521	27.5	*trans*-Calamenene	0.1	MS, I_R
44	1522	1522	27.5	δ-Cadinene	0.2	MS, I_R
45	1574	1577	29.6	Spathulenol	0.3	MS, I_R
46	1579	1582	29.8	Caryophyllene oxide	0.3	MS, I_R
47	1612	1618	31.0	1,10-di-epi-Cubenol	0.2	MS, I_R
48	1639	1638	32.0	Epi-α-Cadinol	0.2	MS, I_R
49	1652		32.5	n.i.	0.4	
50	1686	1688	33.7	Shyobunol	0.1	MS, I_R
Grouped components (%)						
Total monoterpene hydrocarbons	26.64					
Total sesquiterpene hydrocarbons	11.07					
Total oxygenated monoterpenes	60.11					
Total oxygenated sesquiterpenes	1.07					
Total identified	98.89					

Notes: [a]I_R = Linear Retention Index (10); [b]$I_{R(litt)}$ = Linear Retention Index from literature (8, 9); [c]Identification has been realized by comparing mass spectra (MS), retention Indices (I_R); n.i., not identified. Compounds are listed in order of their elution from a HP-5 column.

membrane of cells to reduce the damage caused by free radical attack on erythrocytes.

To the best of our knowledge, the reducing power, phosphomolybdenum assay and KRL biological test have not been formerly tested for *Mentha* sp. essential oils. The antioxidant activity of our essential oil is probably due to the presence of the great amount of oxygenated compounds it contains (60.1%).

Antimicrobial activity

The antimicrobial activity of the *M. spicata*'s essential oils against Gram positive and Gram negative bacterial, yeast, and fungal strains is shown in Table 4.

The essential oils from the mint species exhibited good antimicrobial activity. Of note, results obtained by the spots method were relatively higher than those obtained

Table 2. Different antioxidant assays of essential oil extracted from leaves of *M. spicata*.

Sample	DPPH(IC_{50})	ABTS(IC_{50})	RP (RP_{50})	Phosphomolybdate (RP_{50})
Oil	9544.6 ± 196.2[b]	36.2 ± 3.2[b]	452. 3 ± 0.4[d]	53.3 ± 2.8[d]
BHT	12. 2 ± 1.5[a]	2. 4 ± 0.4[a]	5. 9 ± 0.1[b]	27.2 ± 0.7[c]
α-tocopherol	7.7 ± 0.7[a]	3. 8 ± 0.4[a]	10. 3 ± 0.2[c]	16. 6 ± 0.2[b]
Ascorbic acid	4. 8 ± 1.8[a]	1.8 ± 0.1[a]	2. 8 ± 0.2[a]	9. 6 ± 0.3[a]
BHA	6. 6 ± 0.1[a]	1.7 ± 0.1[a]	2.4 ± 0.1[a]	10.3 ± 0.4[a]

Notes: 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, Reducing Power (RP), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), IC_{50} and RP_{50} values were expressed in µg/mL.

Table 3. Kit Radicaux Libres (KRL) biological test.

Augmentation of control blood half-haemolysis time (%)		Equivalent Trolox® mg/g of oil		Equivalent gallic acid mg/g of oil	
100 mg/L	50 mg/L	100 mg/L	50 mg/L	100 mg/L	50 mg/L
4.0 ± 1.1	7.0 ± 0.4	20.0 ±5.4	69.6 ± 3.6	8.5 ± 2.3	29.5 ±1.5

by the disc diffusion agar method. However, the variation in antimicrobial activities of *M. spicata* essential oils with respect to methods was not statistically significant ($P < 0.05$).

As can be seen in Table 4, undiluted essential oil of *M. spicata* was found to have an appreciable antibacterial activity. However, this oil showed no effect on *Pseudomonas aeruginosa* ATCC 27853. Similarly, Boukhebti et al. (21) found that *Pseudomonas aeruginosa* ATCC 27853 was resistant to the essential oil of *M. spicata* from another location of Algeria (Setif).

The antibacterial activity is more important in some cases, like in the cases of controls, penicillin and streptomycin. Results obtained in this study show that the antibacterial activity of spearmint essential oil is not selective on the basis of the cell-wall differences of bacterial microorganisms (Gram-positive or Gram-negative bacteria) as reported previously (24).

Essential oil of *M. spicata* exhibited antimicrobial activity against *Aspergillus flavus* NRRL 391 and *Candida albicans* ATCC 1024 (zone diameters > 40 mm). These results are in accordance with those reported by Mkaddem et al. (24). They found that the diameter of inhibition obtained for *M. spicata* essential oil against *Aspergillus ochraceus* NRRL 3174 was 43 mm. Furthermore, *Aspergillus niger* was the most sensitive strain among the plant pathogenic fungi tested by Hussain et al. (23).

According to Hussain et al. (23), *M. spicata* exhibited good antimicrobial activity with inhibition zones of 12–29 mm and 16–29 mm against bacterial and fungal strains, respectively. Our results are also in accordance with the findings of Kizil et al. (26) who reported that *M. spicata*'s essential oil exhibited good antimicrobial activity against a wide range of microorganisms. Essential oils rich in compounds of known antimicrobial activities such as carvone are widely reported to possess high levels of antimicrobial activity (23).

Insecticidal activity

Essential oils can affect insects in several ways: they may disrupt major metabolic pathways and cause rapid death, act as fumigants, and/or contact insecticides and repellents (27). The results from this study indicate that the essential oil of *M. spicata* exhibited effective toxicity to *R. dominica* adults in all aspects (contact and fumigation bioassays and repellency).

In the contact toxicity test, results from topical applications showed that the essential oils had a low insecticidal activity in a dose-dependent manner (Figure 2A). The LD_{50} value was calculated as 6.1 µL/mL with 95% confidence limits from 0.5 to 2.0µL/mL and the regression equation was obtained from the regression line. The contact toxicity of *M. spicata* oil admixed in grain was low even at the dose of 2 µL/mL (14.1%). This may be due to the absorption of *M. spicata* oil by the grain. According to Tripathi and Upadhyay (28), plant essential oils were less effective in the presence of a stored product, such as wheat, than in its absence.

Benyoussef et al. (29) showed that toxicity by contact of *M. spicata* essential oils against *Rhyzopertha dominica* is a result of synergical effect of 1,8-cineole and carvone. The amount of 1,8-cineole determined in this study was low which was probably responsible for the weak toxicity effect noted by contact. Moreover, the persistence of the insecticidal activity of essential oils is influenced by the sensitivity of the target pest to their active constituents (28).

The fumigant toxicity of the *M. spicata* oil recorded was moderate. There was an increase in the insect mortality with concentrations of used solutions of essential oils as shown in Figure 2A. At a concentration of 2 µL/mL, the mortality rate was 43% after 96 hours of treatment.

Table 4. Antimicrobial activity (diameter of the inhibition zones in mm) of *Mentha spicata* essential oils.

		Essential oils inhibition zones[1]								References standard	
		1/1 (v/v)		1/2 (v/v)		1/5 (v/v)		1/10 (v/v)			
	M.O	DM	SM	DM	SM	DM	SM	DM	SM	Penicillin	Streptomycin
Fungi	*A. niger* 2CA 936	32.0 ± 1.0^{a}	36.0 ± 1.0^{b}	9.0 ± 0.0^{a,b}	16.0 ± 2.0^{b}	8.0 ± 0.0^{b}	14.7 ± 1.5^{b}	6.0 ± 0.0^{b}	10.7 ± 0.6^{b}	nd	nd
	A. flavus NRRL 391	36.0 ± 2.0^{a}	43.7 ± 0.6^{a}	6.0 ± 0.0^{d}	7.0 ± 1.7^{d}	6.0 ± 0.0^{c}	7.3 ± 1.1^{d}	6.0 ± 0.0^{b}	7.0 ± 1.0^{d}	nd	nd
Yeast	*C. albicans* ATCC 1024	23.3 ± 0.6^{b}	44.3 ± 1.1^{a}	6.0 ± 0.0^{d}	19.0 ± 1.0^{a}	6.0 ± 0.0^{c}	16.3 ± 0.5^{a}	6.0 ± 0.0^{b}	14.3 ± 1.5^{a}	nd	nd
Gram positive bacteria	*MRSA* ATCC 43300	24.0 ± 1.0^{b}	22.3 ± 1.5^{c}	7.0 ± 0.0^{c,d}	7.0 ± 0.0^{d}	7.0 ± 0.0^{c}	7.0 ± 0.0^{d}	7.0 ± 0.0^{a,b}	7.0 ± 0.0^{d}	20.0 ± 1.0^{c}	23.6 ± 0.6^{b}
	B. subtilis ATCC6633	17.7 ± 0.6^{c}	32.7 ± 0.6^{b}	8.3 ± 0.6^{b}	6.0 ± 0.0^{d}	8.0 ± 1.0^{b}	6.0 ± 0.0^{d}	7.7 ± 0.6^{a}	6.0 ± 0.0^{d}	37.3 ± 0.6^{b}	nd
	S. aureus NCCB 9163	14.3 ± 1.5^{d}	20.3 ± 0.6^{c,d}	6.0 ± 0.0^{d}	6.0 ± 0.0^{d}	6.0 ± 0.0^{c}	6.0 ± 0.0^{d}	6.0 ± 0.0^{b}	6.0 ± 0.0^{d}	45.5 ± 0.7^{a}	21.0 ± 0.0^{c}
Gram negative bacteria	*E. coli* ATCC 25922	11.0 ± 1.0^{e}	22.0 ± 1.0^{c}	8.0 ± 1.7^{b,c}	11.3 ± 0.6^{c}	8.0 ± 1.7^{b}	10.7 ± 1.1^{c}	8.0 ± 1.7^{a}	8.7 ± 1.1^{c}	9.0 ± 1.0^{d}	20.3 ± 0.6^{c}
	P. aeroginosa ATCC27853	6.0 ± 0.0^{f}	6.0 ± 0.0^{d}	6.0 ± 0.0^{d}	6.0 ± 0.0^{d}	6.0 ± 0.0^{c}	6.0 ± 0.0^{d}	6.0 ± 0.0^{b}	6.0 ± 0.0^{d}	nd	20.3 ± 0.6^{c}
	K. pneumoniae E47	10.3 ± 0.6^{e}	17.3 ± 0.6^{d}	9.7 ± 0.6^{a}	10.7 ± 0.6^{c}	9.3 ± 0.6^{a}	10.3 ± 0.6^{c}	7.3 ± 0.6^{a}	9.3 ± 0.6^{c}	nd	36.0 ± 3.5^{a}

Notes: *M.O, microorganisms; MRSA, Methicillin-resistant Staphylococcus aureus; S. aureu, Staphylococcus aureus; B. subtilis, Bacillus subtilis; E. coli, Escherichia coli; P. aeroginosa, Pseudomonas aeroginosa; K. pneumonia, K. pneumonia; A. niger, Aspergillus niger; A. flavus, Aspergillus flavus NRRL; C. albicans, Candida albicans;* SM, spots method; DM, disc method; nd, not determined. [1]Values (Diameter of inhibition zone (mm) including disc diameter of 6 mm) are mean ± standard deviation of three different samples of *M. spicata* essential oil, analyzed individually in triplicate. Means followed by different letters in the same row represent significant difference ($P < 0.05$).

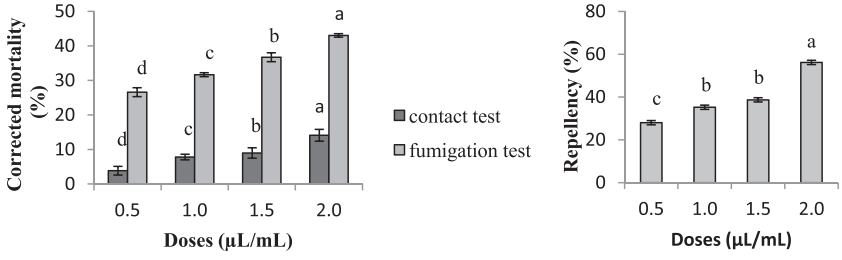

Figure 2. Insecticidal activity of *M. spicata* essential oil against *Rhyzopertha dominica* (F.) adults. Percentage mortality after 96 hours of treatment (A) in fumigation and contact bioassays (B) in repellency bioassay. Note: Each data point represents mean ± SD of four replicates, each comprising twenty adult insects. Data were analyzed using one-way ANOVA (analysis of variance). Different letters above the bars for the same concentration indicates significant differences among means of treatments ($P < 0.05$).

The possible mode of fumigant action may be attributed to octopamine receptor blockage and respiratory arrest (28).

M. spicata essential oils showed a dose-dependent repellency against *R. dominica* adults (Figure 2B). It was evident that, at the highest dose (2 µL/mL), *M. spicata* essential oils exhibited a higher repellent effect (56. 2%). No significant differences were observed for the repellent effect of the 1 and 1.5 µL/mL doses, thus leading it to the repellency class III (Moderately Repulsive), according to Juliana and Su (16).

We demonstrated for the first time in this study that *M. spicata* essential oils could act as fumigants and repellents against *R. dominica* adults. Their repellent and/or insecticidal effects could be used to prevent insect infestations of cereal products by incorporating an appropriated amount into packaging materials. However, large-scale trials are necessary to implement an application method of essential oils against stored product insect pests.

Conclusions

This study has been concerned with determining the chemical composition characteristics of essential oils extracted from *M. spicata*, collected in Kabily (Bejaia, Algeria). It also assembles their antimicrobial, antioxidant and insecticidal activities. Our data confirm and extend, using two methods, the previously reported inhibitory activity against bacteria and fungi of *M. spicata* essential oils. We noted, by comparing different antioxidant methods, that *M. spicata* essential oils possess remarkable radical-scavenging activity towards $ABTS^{\bullet+}$ and a moderate reducing power capacity. Investigation of insecticidal activity against *R. dominica* established that the essential oils possess a contact-fumigant toxicity and a positive repellent activity against adults of *R. dominica*. As a whole, these findings may confirm the interesting potential of this plant as a valuable source of natural bioactive molecules.

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Disclosure statement

No potential conflict of interest was reported by the authors.

References

1. L. Rodrigues, O. Povoa and G. Generosa Teixeira, A. C. Figueiredo, M. Moldao and A. Monteiro, *Trichomes micromorphology and essential oil variation at different developmental stages of cultivated and wild growing Mentha pulegium L. populations from Portugal*. Ind. Crop. Prod., **43**, 692–700 (2013).
2. F. Brahmi, K. Madani, F. Dahmoune, T. Rahmani, K. Bousbaa, S. Oukmanou and M. Chibane, *Optimisation of solvent extraction of antioxidants (phenolic compounds) from Algerian mint (Mentha spicata L.)*. Phcog. Commn., **2 (4)**, 72–86 (2012).
3. P. Arumugam, P. Ramamurthy, S.T. Santhiya and A. Ramesh, *Antioxidant activity measured in different solvent fractions obtained from Mentha spicata Linn.: an analysis by ABTS*+ decolorization assay*. Asia. Pac. J. Clin. Nutr., **15** 119–124 (2006).
4. H. Oumzil, S. Ghoulami, M. Rhajaoui, A. Ilidrissi, S. Fkih-Tetouani, M. Faid and A. Benjouad, *Antibacterial and antifungal activity of essential oils of Mentha suaveolens*. Phytother. Res., **16**, 727–731 (2002).
5. I. Telci, I. Demirtas, E. Bayram, O. Arabaci and O. Kacar, *Environmental variation on aroma components of pulegone/piperitone rich spearmint (Mentha spicata L.)*. Ind. Crop. Prod., **32**, 588–592 (2010).
6. R. Scherer and M. Fumiere Lemos, G. Coimbra Martinelli, J.D. Lopes Martins and A.G. da Silva, *Antioxidant and antibacterial activities and composition of Brazilian spearmint (Mentha spicata L.)*. Ind. Crop. Prod., **50**, 408–413 (2013).
7. A. Kedia, B. Prakash, P. Kumar Mishra, C.S. Chanotiya and N. Kishore Dubey, Antifungal, antiaflatoxigenic, and insecticidal efficacy of spearmint (*Mentha spicata* L.) essential oil. Int. Biodeter. Biodegr., **89**, 29–36 (2014).
8. NIST/EPA/NIH, *Mass Spectral Library*, National Institute of Standard and Technology, Gaithersburg, MD (2005).
9. R.P. Adams, *Identification of essential oil components by Gas Chromatography/ Mass Spectroscopy*, 4th ed.., Allured Publ. Corp, Carol Stream, IL (2007).
10. H. Van Den Dool and P.D. Kratz, *A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography*. J. Chromatogr., **11**, 463–471 (1963).
11. M.S. Blois, *Antioxidant determination by the use of stable free radicals*. Nature., **26**, 1199–1200 (1958).
12. R. Re and N. Pellegrini, A. Proteggente, A. Pannala, M. Yang and C. Rice-Evans, *Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free. Radic*. Biol. Med., **26**, 1231–1237 (1999).
13. M. Oyaizu, *Antioxidant activity of browning products of glucosamine fractionated by organic solvent and thin-layer chromatography*. Nippon Shokulin Kogyo Gakkaishi., **35**, 771–775 (1986).
14. R. Rossi, C. Corino, G. Pastorelli, P. Durand and M. Prost, *Assessment of antioxidant activity of natural extracts*. Ital. J. Anim. Sci., **8**(2), 655–657 (2009).
15. B. Conti, A. Canale, P.L. Cioni, G. Flamini and A. Rifici, *Hyptis suaveolens and Hyptis spicigera (Lamiaceae) essential oils: qualitative analysis, contact toxicity and repellent activity against Sitophilus granarius (L.) (Coleoptera: Dryophthoridae)*. Pest. Sci. J., **84**, 219–228 (2011).
16. G. Juliana and H.C.F. Su, *Laboratory studies on several plant materials as insect repellents for protection of cereal grains*. J. Econ. Entomol., **76**, 154–157 (1983).
17. W.S. Abbott, *A method of computing the effectiveness of an insecticide*. J. Econ Entomol., **18**, 265–267 (1925).
18. G. Kofidis, A. Bosabalidis and S. Kokkini, *Seasonal variation of essential oils in a linalool-rich chemotype of Mentha spicata grown wild in Greece*. J. Essent Oil Res., **16**, 469–472 (2004).
19. J.U. Chowdhury, N.C. Nandi, M. Uddin and M. Rahman, *Chemical constituents of essential oils from two types of spearmint (Mentha spicata L. and M. cardiaca L.) introduced in Bangladesh. Bangladesh*. J. Sci. Ind. Res., **42**, 79–82 (2007).
20. N.M. Mkolo, J.O. Olowoyo, K.B. Sako, S.T.R. Mdakane, M.M.A. Mitonga and S.R. Magano, *Repellency and toxicity of essential oils of Mentha piperita and Mentha spicata on larvae and adult of Amblyomma hebraeum (Acari: Ixodidae)*. Sci. J. Microbiol., **1**, 1–7, (2011).
21. H. Boukhebti, A.N Chaker, H. Belhadj, F. Sahli, M. Ramdhani, H. Laouer and D. Harzallah, *Chemical composition and antibacterial activity of Mentha pulegium L. and Mentha spicata L. essential oils*. Der Pharmacia Lettre., **3**, 267–275 (2011).
22. H. Allali, I. Chikhi, M.E. Dib, A. Muselli, N. Fekih, N. Meliani, M.A. Kamal, B. Tabti and J. Costa, *Antioxidant activity and chemical analysis of Mentha spicata cultivated from west northern region of Algeria by headspace solid phase micro-extraction and hydro-distillation*. Nat Prod I. J., **9(6)**, 258–263 (2013).
23. A.I. Hussain, F. Anwar, P.S. Nigam, M. Ashraf and A.H. Gilani, 2010. *Seasonal variation in content, chemical composition and antimicrobial and cytotoxic activities of essential oils from four Mentha species*. J Sci Food Agric., **90**, 1827–1836 (2010).
24. M. Mkaddem, J. Bouajila, M. Ennajar, A. Lebrihi, F. Mathieu and M. Romdhane, *Chemical composition and antimicrobial and antioxidant activities of Mentha (longifolia L. and viridis) essential oils*. J. Food. Sci., **74**, 358–363 (2009).
25. D. Zhao, Y.W. Xu, G.L. Yang, A.M. Husaini and W. Wu, *Variation of essential oil of Mentha haplocalyx Briq. and Mentha spicata L. from China. Ind Crop*. Prod., **42**, 251–260 (2013).
26. S. Kizil, N. Hasimi, V. Tolan, E. Kilinc and U. Yuksel, *Mineral content, essential oil components and biological*

activity of two Mentha species (M. piperita L., M. spicata L.). Turk. J. Field Crops, **15**(2), 148–153 (2010).

27. R. Islam and R. Islam Khan, S., AI-Reza, Y.T. Jeong, C.H. Song and M. Khalequzzaman, *Chemical composition and insecticidal properties of Cinnamomum aromaticum (Nees) essential oil against the stored product beetle Callosobruchus maculates (F.)*. J. Sci. FoodAgri., **89**, 1241–1246 (2009).
28. A. Tripathi and S. Upadhyay, *Repellent and insecticidal activities of Hyptis suaveolens (Lamiaceae) leaf essential oil against four stored-grain coleopteran pests*. Int. J. Trop. Insect. Sci., **9**(4), 219–228 (2009).
29. H. Benyoussef, O. Khalfi and N. Yahiaoui, *Extraction, analysis and insecticidal activity of spearmint essential oil from Algeria against Rhyzopertha dominica* (F.). J. Essent. Oil. Bear Pl., **9**(1), 17–21 (2006).