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Chemical Composition, Antibacterial and Antioxidant Activities of the Essential Oil Extracted from the *Mentha piperita* of Southern Algeria

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ABSTRACT

The aim of this study is to investigate for the first time the chemical composition of the essential oil extracted from *Mentha piperita* cultivated in Ouargla, Algeria. The other objective is to determine the antioxidant and antibacterial activities against certain bacterial strains in order to find new metabolite products, which are characterized by a biological activity. Twenty-three compounds were identified representing 99.99% of the total oil. The principal components are: Carvone (51.04%), Limonene (36.37%) and β-Pinene (1.66%), which compose 89.07% of the oil. The antimicrobial activity of oil was tested using the diffusion method by determining the inhibition zone and the minimal inhibitory concentration. The results showed a great potential of antimicrobial activity against the bacterial strains with very high degree of sensitivity of *E. coli* Gram-negative strains than other bacterial tested. Furthermore, the essential oil was tested for its antioxidant activity using the 2,2-diphenyl-l-1-picrylhydrazyl (DPPH) radical-scavenging and Ferric Reducing Power (FRAP) methods. The result showed a considerable level of antioxidant activities of the essential oil investigated with (IC_{50} = 32.94 μg mL^{-1}) lower than synthetic antioxidant agents ascorbic acid for DPPH method and higher than synthetic antioxidant agents for Ferric reducing power method with EC_{50} value of 48,76 μg mL^{-1}.

Key words: Antibacterial activity, antioxidant activity, biopesticides, chemical composition, essential oil, GC/MS, *Mentha piperita*

INTRODUCTION

The history of medicinal and aromatic plants is associated with the evolution of civilization. In all regions of the world, the history of nations shows that these plants have always occupied an important place in medicine, perfume composition and culinary preparations (Yakhlef *et al*., 2011).

The valorization of these natural plant resources is essentially by extracting their Essential Oils (EO). Essential oils and their components are started and continuing to have a lot of interest as a potential source of natural bioactive molecules; they are being studied for their possible use as an alternative for treatment of infectious diseases and food protection against oxidation (Bouhdid *et al*., 2006; Goudjil *et al*., 2015).

Currently, essential oils are used in aromatherapy, pharmacy, perfumery and cosmetics (Kanko *et al*., 2004), due to their wealth through active components that are loaded by a vital energy of natural source.

In Algeria, there is an important plants diversity due to the wide variety of biotopes related to climate differences that give the chances to find new alternative natural active for synthetic antibiotic or food conservation.

The family of Lamiaceae contains an extremely wide variety of aromatic plants; they have a high importance because of their use in folk medicine. The genus of Mentha (Lamiaceae) kind represented by 20 species in the world (Derwich *et al*., 2011). This genus is widely cultivated in Sahara of Algeria, where its represented by six spices scilicet: *M. aquatic*, *M. spicata*, *M. rotundifolia*, *M. longifolia*, *M. pulegium* and *M. piperita* (Ladjel *et al*., 2011).

Peppermint (*Mentha piperita*) one of those plants that is native of Middle East. It is the result of hybridization between water mint (*Mentha aquatica*) and spearmint (*Mentha spicata*) (Iscan *et al*., 2002; Kizil *et al*., 2010). In traditional medicine, it is used for its antiseptic, antiviral, antispasmodic, antibacterial and antioxidant activity (Derwich *et al*., 2011; Charles, 2013).

The present study aimed to define for the first time the Algerian chemo-type of the investigated specie growing in arid climatic conditions with high temperature and valorize it essential oil as antioxidant and antimicrobial agent.

MATERIALS AND METHODS

Vegetal material: Leaves of *Mentha piperita* were collected on June, 2013 in the region of Ain Moussa in Ouargla (Algeria), where the climate in desert is very dry with a high temperature which reached at that time (40°C). The gps coordinates are (N 36°52'18.011" E 5°20'36.7"). After collection, the leaves were dried in the shade for five days. The plant has been identified by Dr. Abdelmadjid Chahma a botanist in Biology Department, Ouargla University, Algeria, a specimen was deposited at the herbarium of the University under the number GO2013-1.

Extraction of essential oil: The extraction of essential oil was carried by steam distillation, in a Clevenger apparatus by immersing 100 g of dry leaves in a flask of 1000 mL of water for 3 h. The obtained essential oil was dried with $MgSO_4$ and stored in the dark at 4°C.

Gas chromatography-mass spectrometry analysis of essential oil: The essential oil was analyzed in INRAP (national institute of research and physico-chemical analysis) Tunisia, by using an Agilent 6890 gas chromatograph coupled to an Agilent 5975B mass selective detector with electron impact ionization (70 eV) and an Agilent Chemstation software (Agilent Technologies, Palo Alto, USA). Separation of oil components was performed on HP-5MS; 5% Phenyl Methyl Siloxane capillary column (30 m×0.25 mm, film thickness 0.25 μm) in the split mode (1:50) at 250°C. The oven temperature was set at 50°C for 1 min, then raised to 300°C at 2°C min^{-1} and finally held at this temperature for 10 min. Helium was used as carrier gas at a flow of 0.8 mL min^{-1}. Linear Retention Indices (RI) for all compounds were determined using n-alkanes as standards. Identification of individual compounds was performed by matching their mass spectral fragmentation patterns with corresponding data (NIST 05 and Wiley 7 libraries) and by the laboratory database.

Antibacterial activity

Bacterial strains used: Microbiological material consists of six pathogenic bacterial strains responsible for some more or less serious infectious diseases. They have been provided by

microbiology laboratory Mohamed Boudiaf ouargla hospital and the microbiology laboratory of the University Kasdi Merbah, Ouargla. These bacteria are: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Staphylococcus* sp., *Klebsiella pneumonia*, *Proteus* and *Escherichia coli*.

Disc diffusion method: The evaluation of the antibacterial activity of *M. piperita* essential oil was performed using the disc diffusion method according to the NCCLS recommendations (NCCLS., 2000).

The disc method is a technique of distributing products to test from a paper disc that can qualitatively measure the sensitivity of antimicrobial effects (Aouni *et al*., 2013).

This method has been chosen in this study for its reliability and simplicity. It will provide preliminary results on the sensitivity of the strains and antibacterial activities of the product through the diameters of inhibition appearing around the discs.

The bacterial strains was spread on the Mueller Hinton Agar (MHA). Discs (6 mm Ø; Whatman No. 3) impregnated in the essential oil were placed on the surface of such media and incubated at 37°C for 24 h. All assays were performed three times.

Determination of the Minimum Inhibitory Concentration (MIC): The Minimum Inhibitory Concentration (MIC) of oil was determined for microbial strains by disk diffusion in Mueller-Hinton according to Benabderrahmane *et al*. (2009) with some modification. Because of the non-miscibility of essential oils in water, this latter was diluted in DMSO (dimethyl sulfoxide) to get a concentration range of 1-0.01 mg mL^{-1} and then incorporated into discs of 6.0 mm diameter by 0.01 mL. The same volume of DMSO was used as control.

The microbial suspensions were calibrated according to the standards (0.5 McFarland equivalent that are (10^8 CFU mL^{-1}), 0.1 mL of inoculum was inoculated into the agar immediately. The discs containing various concentrations of oil were placed directly on the surface of the agar. Durations and incubation temperatures were 24 h and 37°C. The MIC was the lowest concentration of essential oil required to completely inhibit the growth of the tested microorganisms around the disc.

Antioxidant activity

Free radical scavenging effect: Antiradical activity of *M. piperita* essential oil was evaluated by measuring the scavenging activity on the 2,2-diphenyl-l-1-picrylhydrazil (DPPH) radical, using the method described by Braca *et al*. (2002) and Mighri *et al*. (2010), with slight modification. A various dilutions of essential oil and DPPH radical solution was prepared in absolute ethanol. One milliliter of each sample concentration was mixed with a same volume of 0.1 mM DPPH. The reaction was carried out at room temperature in the dark for 30 min and after that, the absorbance was recorded at 517 nm. Mixture of 1 mL of DPPH solution and 1 mL of ethanol was taken as a blank. Ascorbic acid was used as a positive control. Inhibition of DPPH free radical in percent (I%) was estimated as follows:

$$I(\%) = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100$$

where, A_{blank} is the absorbance of the control reaction and A_{sample} is the absorbance of the test essential oil or Ascorbic acid. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract concentration. Tests were carried out in triplicate.

Ferric reducing power: The iron reducing activity of our essential oil was determined according to the method described by Oyaizu (1986). Sample of 1.0 mL of various dilutions was mixed with 2.50 mL of phosphate buffer (0.2 mol L^{-1}, pH 6.6) and 2.5 mL of 1% potassium-ferricyanide. The mixture was incubated at 50°C for 20 min. Then, 2.5 mL of 10% trichloroacetic acid was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. After that, 2.5 mL of this mixture was added to 2.5 mL distilled water and 0.5 mL ferric chloride (0.1%), vigorously mixed, finally the absorbance was measured at 700 nm. Essential oils were diluted in Ethanol. Blank sample, similarly prepared by replacing extracted with ethanol, which is used to calibrate the instrument (UV-VIS spectrophotometer). Ascorbic acid (in the 10-100 μg mL^{-1} range) was used as a positive control where the absorbance was measured in the same conditions as the samples. The EC_{50} value is the effective concentration at which the absorbance was 0.5 for reducing power and was obtained by interpolation from linear regression analysis (Piaru *et al*., 2012). Increased absorbance of the reaction mixture indicated an increased reducing power (Singh *et al*., 2011).

RESULTS AND DISCUSSION

Gas chromatography-mass spectrometry analysis of essential oil: This is the first report on *Mentha piperita* volatile oil cultivated in Ouargla, Algeria. The plant gave yield of 0.84% of extracted essential oil, with yellowish color and a very strong and persistent odor of mint.

The chemical composition of the essential oil, including the retention index and the percentage relative to each constituent, is presented in Table 1.

Table 1: Chemical composition of *Mentha piperita* essential oil from Ouargla (Algeria)

Compounds	RI	Area (%)
α-thujene	705	0.13
α-pinene	763	1.07
Camphene	819	0.2
β-terpinene	933	0.96
β-pinene	943	1.66
β-myrcene	1023	1.5
Limonene	1241	36.37
Iso-amyl-2-methyl butyrate	1621	0.12
Cis limonene oxide	1801	0.2
Trans-limonen oxide	1831	0.18
Borneol	2002	0.27
L-borneol	2017	0.45
Trans-dihydrocarvone	2206	1.52
Cis-carveol	2461	0.46
Carvone	2602	51.04
Cis-carvone oxide	2741	0.15
Piperitenone	3119	0.47
Piperitenone oxide	3283	0.74
β-bourbenene	3365	0.3
Caryophyllene	3571	0.37
γ-muurolene	4144	0.17
Caryophyllene oxide	4526	0.82
α-cadinol	4868	0.84
Total		99.99

RI: Retention indices relative

A total of Twenty-three compounds were identified, representing approximately 99.99% of the total chemical composition. The major compounds in the essential oil obtained are: Carvone (51.04%), Limonene (36.37%) and β-Pinene (1.66%). According to previous reports regarding this specie, this combination of major compounds were not reported for *M. piperita* in other regions from the world.

Sartoratto *et al*. (2004), reported that the essential oil leaves of *M. piperita* from Cpqba/ Unicamp (Brazil) is characterized by the dominance of linalool (51.0%), carvone (23.42%) and 3-octanol (10.1%). In others reports, the major compound of *M. piperita* oil from Iran is α-Terpinene (19.7%), Pipertitinone oxide (19.3%) and trans-Carveol (14,5%) (Yadegarinia *et al*., 2006). Carvone (30.5%), 1,8-cineole (14.7%) and Menthone (5.2%) was the composition of the leaf oil of *M. piperita* grown in Federal District, Brazil (Grisi *et al*., 2006). According to Derwich *et al*. (2011), Menthone (29.01%), Menthol (5.58%) and Menthyl acetate (3.34 %) were the major components in *M. piperita* from Morocco. In Turkey, Kizil *et al*. (2010) was found (+) -Menthol (38.06%), Menthol (35,64%) and Neo-menthol (6.73%) is the major compound of *M. piperita* essential oil.

It is learned that the variation in chemical composition of essential oils could be attributed to the geographical origin of the plant, the extraction technique, harvest time and climatic factors (Smith *et al*., 2005) in part and genetic factors in other part (Figueiredo *et al*., 2008), that should not be excluded in explaining the chemo-variation of essential oils.

Antibacterial activity: The results of microbial sensitivity of essential oil are summarized in Table 2. The values have shown the average of three measurements. The bacteriostatic action as results from the appearance of an inhibition zone around the paper discs impregnated with extract of the study. The diameter of inhibition zone is different from a bacteria to another. As it has been reported in the literature, we considered that the extract has bacteriostatic action if the inhibition exceeds diameter 8 mm (Moreira *et al*., 2005).

Mentha piperita essential oil reacted positively with microbial strains tested. Wide differences are noted to the diameters of inhibition obtained, ranging from 10-21 mm zones. The plant shown some inhibitory activity of microbial growth and that justifies why it is used in the treatment as an antibacterial traditional medicine (Charles, 2013).

Our results show great variability in qualities of oil against different strains. Only strains of Escherichia coli Gram-negative, which is very sensitive than other bacterial strains tested.

Essential oil has been revealed moderate activity against strains of gram-negative *Proteus, Klebsiella pneumoniae* and gram-positive *Staphylococcus aureus*, *Staphylococcus* sp., with a diameter of inhibition (13.33, 13.16, 12.66 and 13.43) mm and minimal inhibitory concentration (0.25, 0.5, 0.66 and 0.5) mg mL^{-1}, respectively.

Table 2: Antibacterial activity of *Mentha piperita* essential oil

Micro-organisms	Disc diffusion assay (inhibition zone mm)	MIC (mg mL^{-1})
Gram-negative		
Escherichia coli	21.47±0.50	0.16
Proteus	13.33±0.76	0.25
Klebsiella pneumonia	13.16±0.29	0.50
Pseudomonas aeruginosa	10.66±1.53	-
Gram-positive		
Staphylococcus aureus	12.66±0.76	0.66
Staphylococcus sp.	13.43±0.12	0.50

Values are given as Mean±SD (n = 3)

The antibacterial activity of natural substances can be explained by lysis of bacterial membranes; essential oils could split the lipids of cell membrane and make them more permeable, leading to leakage of ions and cell contents. Extensive loss of critical molecules and ions will cause death of cells (Nanasombat and Wimuttigosol, 2011).

Strains of *Pseudomonas aeruginosa* are most resistant, this is related to their greater ability to develop resistance vis-a-vis many antimicrobial agents, where their frequent involvement in hospital infections (Mann *et al*., 2000). Several authors report the low sensitivity of *Pseudomonas aeruginosa* strains for essential oil of *Mentha piperita* (Aridogan *et al*., 2002; Sokovic *et al*., 2007). In fact, the resistance of this strain is not surprising; as these bacteria have an intrinsic resistance to biocides, which is related to the nature of their outer membranes whose forming an impermeable barrier to hydrophobic compounds (Mann *et al*., 2000). In the presence of permeabilizing agents of the outer membrane, inactive substances against these bacteria become active.

Significant features responsible for the antimicrobial action of the essential oils include hydrophobic components that enable participation of cell membrane lipids from the bacteria, disrupting the cellular structures and render them more permeable (Silva and Fernandes, 2010). The antimicrobial activity of *M. piperita* oil is due to various chemical agents present in the extract. The activity may be easily ascribed to oxygenated monoterpenes compound carvone and monoterpene hydrocarbons compound limonene that present in high percentage in the oil. However, the optimal efficiency of an extract cannot be due to a major active constituent (chymotypes), but to the combined action (synergy) of different compounds exist in this essential oil.

The sensitivity of medical strains, including those resistant to multiple antibiotics (*Pseudomonas aeruginosa*, Table 2), to essential oil *M. piperita,* suggesting their potential use in therapeutics as a natural alternative to chemotherapeutic agents whose action spectrum is ongoing reduced.

Antioxidant activity: Antioxidant activity of *M. piperita* essential oil was determined by two different methods such as free radical-scavenging assay (DPPH) and ferric reducing power. The result reported in Table 3.

In DPPH, assessed sample was able to reduce the stable violet DPPH radical to the yellow DPPH-H, Reaching 50% of reduction with IC_{50} value of (32.94±2.70 µg mL^{-1}). Compared with synthetic antioxidant agents ascorbic acid (IC_{50} = 6.56±0.21 µg mL^{-1}) and other species of the genus *Mentha* like, *M. spicata* (IC_{50} = 13.3 µg mL^{-1}) from Pakistan (Hussain *et al*., 2010), *M. rotundifolia* (IC_{50} = 26.11 µg mL^{-1}) from Tunisia (Riahi *et al*., 2013), *M. piperita* (IC_{50} = 3.9 µg mL^{-1}), *M. longifolia* (IC_{50} = 24.07 µg mL^{-1}) and *M. spicata* (IC_{50} = 1.14 µg mL^{-1}) from Iran (Souri *et al*., 2008; Sharafi *et al*., 2010), *M. piperita* exhibited weak antioxidant abilities. However our result is higher than those mentioned for *M. piperita* essential oil analyzed in morocco (IC_{50} = 53.67 µg mL^{-1}, (Derwich *et al*., 2011)), in India (IC_{50} = 273 µg mL^{-1}, (Samarth *et al*., 2008), in Egypt (IC_{50} = 59,19 µg mL^{-1}) (Gharib and da Silva, 2013) and in Turkey (IC_{50} = 60,41 µg mL^{-1}) (Kizil *et al*., 2010).

Table 3: Antioxidant activities of Algerian *Mentha piperita* essential oil

	Scavenging activity	
Parameters	DPPH IC_{50} (µg mL^{-1})	FRAP EC_{50} (µg mL^{-1})
Mentha piperita essential oil	32.94±2.70	48.76±1.60
Ascorbic acid	6.42±0.36	66.73±0.37

Values are given as Mean±SD (n = 3)

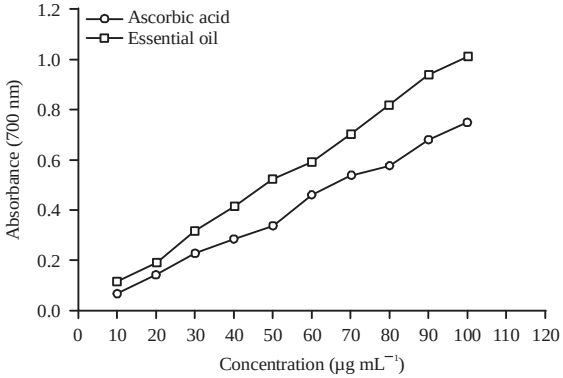

Fig. 1: Reducing power of *Mentha piperita* essential oil

For Ferric reducing power, the presence of reducing agents in the extracts of plants causes the reduction of Fe^{+3}/ ferricyanide complex to the ferrous form. Therefore, Fe^{+2} can be assessed by measuring and follow the increase in the density of the blue color in the reaction medium at 700 nm. *Mentha piperita* essential oil showed antioxidant activity with an EC_{50} value of 48.76±1.60 µg mL^{-1} when ascorbic acid as a positive control showed EC_{50} value of 66.73±0.37 µg mL^{-1}. Figure 1 indicate that, the reducing power increased with the concentration of samples and clearly demonstrated that the essential oil has significant antioxidant activity against control.

CONCLUSION

This study is the first characterization of *Mentha piperita* essential oil growing in Ouargla, Algeria. The investigated oil chemical profile is highlighted by Carvone, limenane and β-Pinene as major compounds. Considerable degrees of antibacterial and antioxidant activities were found in essential oil; evaluated in this study. Our results clearly demonstrate that the essential oils of *Mentha piperita* can well present an interesting alternative naturel, which it can be useful for food preservation and pharmaceutical treatment.

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