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Biological valorization and characterization of essential oil of Algerian *Mentha spicata* L.

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ABSTRACT

Main purpose of our work is to valorise Mentha spicata from the arid zone in Algeria by the study of it biological activities and chemical profile; antibacterial activity and antioxidant activity. Essential oil is characterized by GC-MS. Forty- four constituents, accounting for 98.41% of the total oil contents identified were carbon (42.23%) followed respectively by limonene (29.57%), 1,8-cineole (5.31%), β-pinene (3.54%). The antioxidant activity of the hydro distilled oil was studied using DPPH to determined IC50. The antibacterial activity of essential oils was tested against five microorganisms with the diffusion disc method on bacteria: Staphylococcus aureus ATCC 25923,Candida Albicans ATCC 10231, Escherichia coli ATCC 25922, Bacillus ATCC 11778, Pseudomonas aeruginosa ATCC 27853), diameters vary between 6 and 18 mm.

***Keywords*:** Antibacterial activity, antioxidant activity, chemical composition, essential oil, GC-MS, *Mentha spicata* L.

INTRODUCTION

Aromatics are the origin of metabolites, in particular secondary metabolites with biological effect. The heed on bioactive potential of essential oils and their compounds have been quickly expanded throw last few years (Premathilake *et al.*, 2018). *Our species* is one probably one of the best known and used mint throughout the world (Shahbazi, 2015). It is cultivated in Algeria for its medicinal and culinary application (Brahmi *et al*., 2016). Their leaves are used in the tea making and in some dishes like flavouring (Snoussi *et al*., 2015). *M. spicata* has largly placed on treat numerous pathologies such as some gastrointestinal disorder and also introduced in dental and oral hygiene products (Shahbazi, 2015). The aim of this work is to determinate the phytochemical profile of the essential oil of *Mentha spicata (EOMs)*, growing in the region of the El Bayadh positioned in the south-west of Algeria and to judge their antimicrobial and antioxidant effects.

MATERIALS AND METHODS

Extraction and characterization of essential oil

M. spicata was collected in the month of March 2020 from Brezina (El Bayadh) located in the south-west of Algeria. The aerial parts collected were dried at room temperature. *EOMs* was extracted by hydro distillation utilizing an apparatus of Clevenger type for 4H by mixing 200g of *mint* in1500 ml of distilled water. The evaluation of the chemical profile was carried out by GC-MS analysis performed with a Varian CP-3800 gas chromatograph built with a DB-5 capillary column (30 m × 0.25 mm, 0.25 ìm coating thickness) and a Varian Saturn 2000 ions. The analytical conditions were as follows: injector and transfer line temperatures 220°C and 240°C respectively; programmed over temperature from 60°C to 240°C; helium carrier gas at 1 ml / min; 0.2 µL injection (10% hexane solution); 1:30 division ratio. The identification of the constituents was on the basis of the comparison of retention times with those of authentic samples, comparing their linear retention indices with regards to the hydrocarbon series, and in computer with mass spectra of commercial and household libraries made out of pure substances and the different parts of known oils and data from MS literature data (Adams, 2007; Davies, 1990).

Antioxidant study by DPPH assay

2ml of different concentrations of *M. Spicata* essential was put into 0.4ml of DPPH solution in

ethanol (Blois, 1958). The mixture was kept in the dark for 30 min and was measured at 517 nm. We uses Ethanol as a get a grip on while, Gallic and ascorbic acids were as standards to compare the result.

Free scavenging convenience of DPPH radical was calculated utilizing the following equation: DPPH scavenging effect (%) = [(Absorbance of Control – Absorbance of Sample)/ Absorbance of Control] X 100. The outcomes were recorded as 50% inhibition concentration (IC50).

Antimicrobial study

Microorganisms

The antimicrobial activity of the *EOMs* was evaluated against five strains bacteria, three with Gram negative (*Candida albicans* ATCC 10231, *Escherichia coli* ATCC 25922**,** *Pseudomonas aeruginosa* ATCC 27853), two stains of Gram positive (*Staphylococcus aureus* ATCC 25923, *Bacillus* ATCC 11778)

Test of antibiotics

The antibiotics tested and their corresponding disc concentrations were the following: Trimethoprim 1.25µg + Sulfamethoxazole (**SXT)** 23.75µg, Chloramphenicol (C 30) 30µg, Clindanycin (CMN2) 2µg, Penicillin (PEN) 6µg. The diameter of zone of inhibition was determined using a standard method (Moard, 2008); (Moard, 2011). Plates were incubated for 24h at 37°C

RESULTS AND DISCUSSION

Chemical profile

The chemical composition of the *EOMs* is explained in Table 1. The study of the essential oil resulted in identification of 44 compounds, representing 98.41% of the total, with carbon (42.23%) followed respectively by limonene (29.57%), 1,8-cineole(5.31%), β-pinene (3.54%) β-caryophyllene(2.18%), α-Pinene (1.85%) and germacrene D (1.66%). A similar study of the chemical composition of *EOMs* from Saharan Atlas (Algeria) was made by Sanaa *et al*. (2018). And the same study of *EOMs* from Bejaia (located in Algeria) by Brahmi *et al.*(2016). The composition of this volatile oil is variable according to geographical location, depending on the changing of the climate, and soil, too. The chemical composition can also be modified by the time of the collect and the method of extraction (Laggoune *et al*., 2016).

Antioxidant Activity

The concentration providing 50% effect of DPPHÿ was calculated from the graph of the percentage of scavenging effect of DPPHÿ versus the concentration of the positive control. The results are summarizing in Table 2. In comparison to the conventional compound, the IC50 value for *EOMs* was 24.16 ± 2.12ìg/ml. The IC50 founded through this evaluation was more advanced than those showed by Hussain *et al.* (2010). In an identical study, the IC50 of *EOMs* underneath the IC50 of standards. Snoussi *et al*.(2015) which explain the important antiradical power of the spearmint's essential oil.

Antibacterial activity

Details sensitivity and resistance of the bacteria tested to the different antibiotics which are inside and indicates that the different antibiotics have a more or less similar to that of the Mint essential oils tested after twenty-four hours (Table 3).

Essential oil of spearmint tested against all microorganisms is demonstrated in Table 4. The outcomes of the current survey reveal that the *EOMs* shows an adequate antimicrobial effect from the tested microorganisms. Most Gram-negative bacteria were vulnerable to *EOMs* in addition to Gram-positive bacteria (Table 4). The inhibition zone diameter for *staphylococcus aureus* ATCC 25923 was from 18mm to 5 mm, *Escherichia coli* ATCC 25922 scored growth inhibition of 17mm to 5 mm, for *Candida Albicans* ATCC 10231 18mm to 7mm, for *Bacillus* 18mm to 6mm ATCC 11778 , and *Pseudomonas aeruginosa* ATCC 27853 17 mm to 0mm.

Kindred analysis turned up by Dhifi that the *EOMs* was reactive against Gram- - (*E.coli*) , Gram+ (*s.aureus*) and *Candida Albicans* , with growth diameter about 18 mm against *Escherichia coli,* and 26 mm against *Candida albicans* (Dhifi *et al.,* 2013). At exactly the same path; Rolden *et al.* (2010) reported against *E.coli* ATCC 25922. Mahboubi and Hagi (2008) determinate that the *EOMs* had a top antibacterial activity against *Escherichia coli Bacillus cereus*, and

Table 1: Chemical profile of the *EOMs* aerial parts

	Chemical composition	RI calculated (DB5)	RI Liturature % (Adams 2017)
1	Myrcene	990	0.38
2	α-Terpinene	1013	0.22
3	γ-Terpinene	1063	0.53
4	Limonene	1026	29.57
5	p-Cymene	1022	0.17
6	cis-β-Ocimene	1033	0.57
7	cis-Sabinene hydrate	1100	0.10
8	P-menth-2-en-1-Ol	1120	0.15
9	Terpinen-4-ol	1172	0.99
10	α-Thujone	1101	0.33
11	Eucalyptol = 1,8-cineole	1028	5.31
12	Borneol	1167	0.22
13	Linalool	1093	0.66
14	α-Terpineol	1185	0.15
15	Pulegone	1232	0.32
16	β-Pinene	975	3.54
17	Carvone	1242	42.23
18	Sabinene	967	0.1
19	cis-Carvone oxide	1260	0.10
20	Perillaldehyde	1269	0.11
21	Camphene	945	0.23
22	trans-Carvone oxide	1275	0.13
23	α-Copaene	1372	0.27
24	Bornyle acetate	1286	0.12
25	Camphor	1143	0.27
26	β-Bourbonene	1385	1.53
27	Dihydrocareol acetate	1305	0.17
28	trans-Carvyl acetate	1338	0.10
29	β-Elemene	1387	0.52
30	Eugenol	1355	0.11
31	3-octanol	993	0.65
32	cis-Carvyl acetate	1367	0.10
33	cis-Jasmone	1393	0.38
34	α-Caryophyllene	1455	0.11
35	β-Copaene	1433	0.17
36	Germacrene D	1483	1.66
37	Bicyclogermacrene	1497	0.52
38	α-Pinene	930	1.85
39	Dihydrocarveol	1195	0.44
40	Elemol	1545	0.33
41	β-Caryophyllene	1419	2.18
42	Caryophyllene oxide	1584	0.50
43	α-Thujene	923	0.19
44	β-Thujone	1114	0.13
	Total		98.41

Table 2: Antioxidant activity of *EOMs* by reducing power and DPPH essays

Compound	DPPH (IC50) µg/ml
Quercetin	14.09 ± 1.3a
Gallic acid	6.35 ± 0.41a
Ascorbic acid	7.24 ± 0.97a
Essential oil of M. Spicata	24.16 ± 2.12a

Table 3: Sensitivitiy of pathogen tested on antibiotics

	Zone of inhibition antibiotics (mm)			
	CMN	**SXT 25**	**C 30**	**PEN**
Candida Albicans	29	30	22	35
Staphylococcus aureus	29	21	22	43
Escherichia coli	30	27	25	35
Bacillus	19	30	20	23
Pseudomonas aeruginosa	25	33	28	44

Table 4: Sensitivity tests of bacterial pathogens to mint essential oil.

Pathogen	Zone of inhibition (mm)			
	Mint			
Concentration of *EOMs*				
Candida Albicans	18	15	14	7
Staphylococcus aureus	18	16	13	10
Escherichia coli	17	17	15	6
Bacillus	18	17	7	6
Pseudomonas aeruginosa	17	13	9	-

Staphylococcus aureus, and extended diameter between 8-21mm.

M. spicata acrylic Serbia was examined in the disc diffusion method and demonstrated that its effect against Gram + bacteria was a lot better than Gram- the antibacterial activity. The diameter of the inhibition zone ranges from 10mm to 25mm (Sokovic *et al.,* 2005)**.** Odds-on the antibacterial activity of *EOMs* were recorded to the percentage of oxygenated monoterpenes and monoterpene hydrocarbons (Dahiya and Manglik, 2013)**.** Subsequently, the rich present of carbon, limonene, cineole, can explain the good antibacterial effect that *EOMs* had.

CONCLUSION

The existing research has disclosed the composition of the hydro-distilled *EOMs*, cultivated in the region of El Bayadh (south-west of Algeria), which can be revealed by GC and GC/MS. 44 molecule constituting 98.41% of the *EOMs* and were identified with mains components were carbon, and limonene which can be well matching with the results obtained in other Algerian studies. The *EOMs* showed a higher antioxidant and a moderate to good antimicrobial activities. These biological effects are largely owing to the elevated content of carbon, in this mint.

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