

**Original article:**

**CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY  
OF THE ESSENTIAL OIL FROM LEAVES OF ALGERIAN  
*MELISSA OFFICINALIS* L.**

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**ABSTRACT**

The essential oil obtained from leaves of *Melissa officinalis* L. (Family of Lamiaceae) growing in Algeria, was investigated for its chemical composition and *in vitro* antimicrobial activity. The chemical composition was determined by hydro-distillation and analyzed by GC/MS and GC-FID. Sixty-three compounds were identified in the essential oil, representing 94.10 % of the total oil and the yields were 0.34 %. The major component was geranial (44.20 %). Other predominant components were neral (30.20 %) and citronellal (6.30 %). The *in vitro* antimicrobial activity was determined by paper disk agar diffusion testing and minimum inhibitory concentration (MIC) using 7 bacteria (3 Gram-positive and 4 Gram-negative), 2 yeasts and 3 fungi. The results showed that the essential oil presented high antimicrobial activity against all microorganisms targeted mainly against five human pathogenic bacteria, one yeast *Candida albicans* and two phytopathogenic fungi tested. The minimum inhibitory concentrations (MIC) ranged from 1.00 to 5.00  $\mu$ L/mL.

**Keywords:** *Melissa officinalis*, leaves, essential oil, chemical composition, antimicrobial activity

**INTRODUCTION**

*Melissa officinalis* L. (Lamiaceae) is a perennial edible herb native to the Mediterranean region. The plant is cultivated in various parts of the world and grows especially in western Asia, south-western Serbia and North Africa. In Algeria, this plant is known locally by the names touroudjan, tindjan or bararendjabouya. It is considered as an important medicinal plant largely used in traditional medicine, for the treatment of headaches, indigestion, colic, nervousness, cardiac failure and depression (Beloued, 2009). Actually, essential oils and their components are gaining increasing interest because of

their relatively safe status and their potential use in many functional purposes. The main advantage in the use of such natural agents is that they do not present the phenomenon of drug-resistance, commonly encountered with the long-term use of antibiotics. Their preparations have also found applications as naturally occurring antimicrobial agents in the field of pharmacology, phytopathology and food preservation. *M. officinalis* essential oil is recommended for its antimicrobial activity (Romeo et al., 2008; Hussain et al., 2011; Vitullo et al., 2011; Tullio et al., 2007) and aqueous extracts exhibit antiviral (Adorjan and Buchbauer, 2010; Jassim and Naji,

2003) and antioxidative (Spiridon et al., 2011) properties and anti-inflammatory, antinociceptive (Birdane et al., 2007) and anti-diabetic effects (Chung et al., 2010). The leaves are used as a juice or as a herbal tea for their aromatic, digestive, and antispasmodic properties in nervous disturbance of sleep and for gastrointestinal disorders (Beloued, 2009). It was also reported that *M. officinalis* contains substances inhibiting protein biosynthesis in cancer cells (Adjorjan and Buchbauer, 2010; Carvalho de Sousa et al., 2004). These biological activities have been attributed to the essential oil (Adinee et al., 2008; Da Silva et al., 2005; Sharafzadeh et al., 2007) flavonoids and phenolic acids (Constantine, 2007; Ziakova et al., 2003) such as rosmarinic acid (Toth et al., 2003) and caffeic acids (Tagashira and Ohtake, 1998), phenylpropanoid heteroside (Mulken and Kapetanidis, 1988), Triterpene (Mencherini et al., 2007).

Considering the antimicrobial activity of *M. officinalis* oil, Romeo et al. (2008) and Hussain et al. (2011) reported its antibacterial effect against *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis*, *Bacillus pumilis*, *Pseudomonas aeruginosa*, *Salmonella poona*, *Escherichia coli* and *Listeria innocua*. However, lemon balm oil activity against fungi *Fusarium oxysporum albedinis*, *F. oxysporum lini*, *Mucor ramannianus* and the yeasts *Candida albicans* and *Saccharomyces cerevisiae* has not been reported previously. The chemical composition of Algerian *M. officinalis* oil has not been investigated. Therefore, the aim of this paper is to analyze the chemical composition of hydrodistilled essential oil of *M. officinalis* from Algeria by GC/FID and GC/MS methods, and to investigate its antimicrobial activity against selected human pathogenic and phytopathogenic microbial strains via paper disk agar diffusion method and determination of minimum inhibitory concentrations. A particular interest will be accorded to comparison with oils produced by the same species grown in different regions of the world (Dukic et al., 2004; Holla et al., 1997; Shalaby El-

Gengaihi and Khattab, 1995; Carnat et al., 1998; Adzet et al., 1992; Damien et al., 2000; Sadraei et al., 2003; Basta et al., 2005; Da Silva et al., 2005; Pino et al., 1999). This paper is the first report on the chemical composition and antimicrobial activity of the oil of *Melissa officinalis* growing in Algeria.

## MATERIALS AND METHODS

### Plant materials

The sample of *M. officinalis* was collected in March 2012 at Algiers (Algeria). The plant was identified in the botanical department of National Institute Agronomic of Algiers (NIA), Algeria.

### Chemicals

Myrcene, Linalool, Camphor, Citronellal,  $\beta$ -Caryophyllene, Caryophyllene oxide, citral (isomer of neral and geranial), and the mixture of aliphatic hydrocarbons (C<sub>5</sub>-C<sub>28</sub>) were purchased from Sigma-Aldrich (Germany). All compounds were of analytical standard grade.

### Extraction, isolation and identification of the essential oil

Dried leaves of *M. officinalis* were performed by hydrodistillation for 3 hours using a Clevenger type apparatus. The oil was collected, dried over anhydrous sodium sulphate and stored in the dark at +4 °C until analyzed.

### Physico-chemical indices

The physico-chemical indices of the oil were determined following the ISO regulations. ISO 280:1976 for the refractive index, ISO 279:1981 for the specific gravity, ISO 592:1981 for the optical rotation, ISO 709:1980 for the ester value and ISO 1242:1973 for the acid value.

### Gas chromatography analysis

GC-FID analysis of the volatile components was carried out using a Hewlett Packard HP5890 series II instrument coupled to an ionisation flame detector (FID). Com-

pounds were separated on a HP-5 capillary column (5 % phenylmethylpolysiloxane, 30 m x 0.25 mm i.d., 0.25 µm film thickness) and a HP-WAX (polyethylene glycol, 30 m x 0.15 mm i.d., 0.25 µm film thickness), using the following temperature programme: 5 min at 60 °C, then rising at 3 °C/min to 250 °C, held for 5 min; injector and transfer line temperatures, 250 °C; Azot was used as the carrier gas at a flow rate of 1 mL/min; injection volume, 0.1 µL; split ratio, 1:50. A mixture of aliphatic hydrocarbons (C<sub>5</sub>-C<sub>28</sub>) (Sigma) was directly injected into the GC injector under the above temperature programme in order to calculate the retention index (as Kovats index) of each compound. The percentage composition of the individual components were obtained from electronic integration measurements using flame ionisation detection (FID; 260 °C). n-alkenes were used as reference points in the calculation of retention indices (RI).

#### **GC/MS analysis**

The GC/MS analysis was performed with a Hewlett Packard HP5890 series II gas chromatograph coupled to a HP MSD5971, equipped with an electronic impact source at 200 °C, fitted with a fused silica-capillary column with an apolar stationary phase HP-5MS (5 % phenylmethylpolysiloxane, 30 m x 0.25 mm i.d., 0.25 µm film thickness). The temperature programme conditions were the same with GC analysis. Helium was used as the carrier gas at a flow rate of 1 mL/min; acquisition mass range, 30-600 m/z. All mass spectra were acquired in electron-impact (EI) mode with ionisation voltage of 70 eV.

#### **Identification of the compounds**

The compounds were identified by comparing the retention time, retention index and mass spectrum of the chromatographic peaks with that of the standards. The identification of other volatile components was based on computer matching with the Wiley, NIST and ADAMS libraries (Adams, 2007). Co-injections with authentic samples. Chemicals

were obtained from Sigma-Aldrich chemical, Germany.

#### **Antimicrobial activity**

Antimicrobial activity of the essential oil was screened by the paper disk diffusion method and by the determination of the minimal inhibitory concentrations (MIC).

#### **Microbial strains**

The essential oil and the standard compounds were individually tested against different microorganisms including 3 Gram-positive bacteria (*Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* CIP 7625, *Listeria monocytogenes* CIP82110) and 4 Gram-negative bacteria (*Pseudomonas aeruginosa* CIP A22, *Escherichia coli* ATCC 10536, *Klebsiella pneumoniae* CIP 8291, *Salmonella enterica* CIP 81.3), 3 filamentous fungi (*Fusarium oxysporum albedinis* CURZA, *Fusarium oxysporum lini* CINRA, *Mucor ramannianus* NRRL 6606) and 2 yeasts (*Candida albicans* IPA200, *Saccharomyces cerevisiae* ATCC 4226). All microorganisms were graciously supplied from stock cultures of the Microbiology Laboratory of the Department of Biology, Ecole Normale Supérieure, Algiers, Algeria. The bacterial strains were cultured on Mueller-Hinton agar for 48 h at 37 °C, while fungi and yeasts were propagated on Sabouraud agar at 37 °C for 48 h to 3 days before use. All microorganisms were regenerated twice before use in the manipulations.

#### **Paper disk diffusion assay**

Paper disk-diffusion method (Bauer et al., 1966) was employed for the determination of antimicrobial activity of the essential oil. Microbial suspensions were prepared in sterile 0.9 % saline and adjusted as inoculum to a final concentration of  $1.0 \times 10^8$  CFU/mL. A volume of 20 mL of Mueller-Hinton agar and Sabouraud, respectively, for bacterial and fungal strains was inoculated with 20 µL of microbial suspension and then poured into a Petri dish. The plates were left at room temperature for 30 min to allow the

culture media to solidify. Each paper disk of 6 mm diameter was impregnated with 35 µg of essential oil solution (in methanol) and then applied manually on the surface of the agar plates inoculated with microorganisms. The major components of the essential oil, citral, citronellal and caryophyllene oxide were also tested. Ampicillin and Nalidixic acid (30 µg/disk) were used as positive reference standards to determine the sensitivity of Gram-positive and Gram-negative bacteria species, respectively. Nystatin (30 µg/disk) was used as positive reference standard to determine the sensitivity of fungi and yeasts species. The plates were kept at 4 °C for 2 h to allow diffusion, and then incubated for 24 h at 37 °C for bacteria, and 48 h at 30 °C for yeasts and fungi. The antimicrobial activity was determined by measuring with a ruler, the diameters of inhibition zones, including disk diameter (6 mm). All tests were carried out in triplicate.

#### **Antimicrobial minimal inhibitory concentrations**

The Minimal inhibitory concentrations (MIC) of the essential oil were determined by a conventional agar dilution method (Oki et al., 1990). The microorganisms tests included the same strains of bacteria and fungi used in the screening of antimicrobial activity by the paper disk method. A stock solution of the essential oil was prepared in methanol. The agar media (Mueller Hinton for bacteria and Sabouraud for fungi) were supplemented with different concentrations of essential oil (0.1, 0.2, 0.3, 0.4, 0.5, 1, 2, 3, 4, 5, 8, 10, 20, and 50 µL/mL) and then poured onto Petri dishes. Aliquots of 1 µL of strains suspensions containing 10<sup>8</sup> CFU/mL of the indicator strain were inoculated. A negative control was included in the test by inoculating in the same conditions, the target organisms onto the media without essential oil. The plates were incubated during 24–48 h at 37 °C for bacteria and 48–72 h at 28 °C for fungi. The MICs were determined as the lowest concentration of the essential oil that inhibit the growth of the tested microorgan-

ism, and detected by lack of visual growth, matching with the negative control.

## **RESULTS**

### **Chemical composition analysis**

The oil of the leaves of *M. officinalis* isolated by hydrodistillation was of yellow-pale color with a citron smell, in total yield of 0.34 % w/w on dry weight basis. Some physicochemical characteristics of the oil were also determined:

specific gravity $[d]^{20} = 0.9091$ ;  
refractive index  $\eta^{D20} = 1.3493$ ;  
optical rotation  $\alpha^{D20} = +24.0$ ;  
acid value  $I_A = 1.2$ ;  
ester value  $I_E = 23.59$ .

Qualitative and quantitative analytical results were obtained using both GC and GC-MS techniques. Table 1 shows the compounds identified in the oil of *M. officinalis* in order of elution on HP5 capillary column, the percentage content of the individual components, retention indices and chemical class distribution are summarized.

Thirty six compounds were identified, accounting for 94.10 % of the total oil. This oil was characterized by very high percentage of monoterpenes (84.4 %), especially oxygenated monoterpenes (87.2 %) in which, neral (30.2 %), geranial (44.2 %) and citronellal (6.3 %) were the major components. In contrast, the sesquiterpene fraction was lower (4.8 %); the hydrocarbons (3.5 %) represented by  $\alpha$ -copaene (1.8 %) and  $\beta$ -caryophyllene (1.3 %) were detected in higher concentration than the oxygenated sesquiterpenes, such as caryophyllene oxide (1.3 %).

The above results show that our oil was characterized by the presence of three dominating components in monoterpene family type aldehyds, and an important fraction includes neral (30.2 %), geranial (44.2 %) and citronellal (6.3 %).

### **Results of the antimicrobial tests**

The results of the antimicrobial activities of Algerian *M. officinalis* essential oil per-

**Table1:** Composition of essential oil of *Melissa officinalis* from Algeria

Compounds	R <sub>1a</sub>	R <sub>1p</sub>	%	Identification method
1-Octen-3-ol	979	1421	0.2	GC,GC/MS
6-Methyl-5-hepten-2-one	986	1375	0.6	GC,GC/MS
Myrcene	991	1162	0.1	GC, GC/MS, CO-GC
(Z)-β-Ocimene	1042	1229	0.1	GC,GC/MS
(E)-β-Ocimene	1052	1250	tr	GC,GC/MS
Linalool	1096	1508	0.3	GC, GC/MS, CO-GC
n-Nonanal	1100	1392	0.1	GC,GC/MS
Cis -Rose oxide	1111	1352	0.1	GC,GC/MS
Trans-Rose oxide	1128	1370	0.1	GC,GC/MS
Trans –Limonene oxide	1138	-	tr	GC,GC/MS
Camphor	1143	1518	0.4	GC, GC/MS, CO-GC
Citronellal	1152	1137	6.3	GC, GC/MS, CO-GC
Cis-Chresontynol	1163	-	1.7	GC,GC/MS
Menthol	1174	1612	0.3	GC,GC/MS
Isomenthol	1181	1666	2.4	GC,GC/MS
Nerol	1228	1758	0.2	GC,GC/MS
Neral	1238	1227	30.2	GC, GC/MS, CO-GC
Piperitone	1253	1731	0.1	GC,GC/MS
Geraniol	1258	1837	0.6	GC, GC/MS
Geranial	1271	1732	44.2	GC, GC/MS, CO-GC
Dihydrocitronellol acetate	1319	1670	0.3	GC,GC/MS
α-Cubebene	1352	1361	tr	GC,GC/MS
Geranyl acetate	1364	1753	tr	GC,GC/MS
α-Copaene	1379	1493	1.8	GC,GC/MS
β-Cubebene	1390	1541	0.1	GC,GC/MS
β-Caryophyllene	1416	1617	1.3	GC, GC/MS, CO-GC
α-Humulene	1450	1673	0.2	GC,GC/MS
Germacrene D	1478	1711	tr	GC,GC/MS
(E)-β-Ionone	1482	1918	tr	GC,GC/MS
Valencene	1491	1751	0.1	GC,GC/MS
(E)-Nerolidol	1560	2044	tr	GC,GC/MS
Caryophyllene oxide	1578	2000	1.3	GC, GC/MS, CO-GC
1-Hexadecene	1592	1600	tr	GC,GC/MS
14-Hydroxy-9-epi-(E) Caryophyllene	1663	2193	tr	GC,GC/MS
n-Eicosane	2004	2000	0.6	GC,GC/MS
n-Heneicosane	2108	2100	0.4	GC,GC/MS
Monoterpene Hydrocarbons			0.2	
Oxygenated Monoterpenes			87.2	
Sesquiterpene Hydrocarbons			3.5	
Oxygenated Sesquiterpenes			1.3	
Others			1.9	
Total			94.1	

Order of elution and percentage are given on (HP5MS) column. R<sub>1a</sub>, R<sub>1p</sub> = Retention; Indices on apolar (HP5MS) and polar (HPwax) columns, respectively. tr = trace (<0.1 %); CO-GC, Co-injections with authentic samples

formed by paper disk method and determination of MICs are reported in Tables 2 and 3 respectively. The target microorganisms are considered as among human pathogenic strains (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella enterica*) and common foodborne pathogen (*Listeria monocytogenes*). The essential oil exhibited a strong activity against all the strains tested with very low MICs. The inhibition zones ranged between (17 and 18 mm) for the Gram-positive bacteria, and (14-21 mm) for the pathogenic Gram-negative bacteria. Generally, the essential oils are more active against Gram-positive bacteria than against Gram-negative ones. In our case, *Melissa officinalis* oil is more active against the Gram-negative ones.

The antiyeast activity was potent against the human pathogenic *Candida albicans* (36 mm). The two phytopathogenic fungi tested, *Fusarium oxysporum albedinis* and *Fusarium oxysporum lini* are the agents of vascular wilt (fusariosis) of date palm and flax, respectively. These fungi cause important deteriorations to different parts of the affected plants, including fruits. The results of antifungal activity showed that the essential oil (34-38 mm) was more potent than nystatin (20-24 mm), the reference standard used (see Table 2). These important activities may be related to the major compounds (citral, citronellal and caryophyllene oxide), which provided inhibition zones similar to that of the oil (see Table 2). The resulted whole activity involves probably some type of synergism between many active compounds. *Melissa officinalis* essential oil contains appreciable amounts of oxygenated compounds (monoterpenes and sesquiterpenes) (Table 1). This result is in agreement with the great diameters of inhibition obtained for all the target microorganisms. The values of minimal inhibitory concentrations (MICs) are very low. They ranged from 1 to 5  $\mu\text{L}/\text{mL}$ , indicating and confirming the potent antimicrobial activity of the oil (see Table 3). The

highest MIC was obtained with *Salmonella enterica*, the most resistant strain.

## DISCUSSION

The composition of the oil from *M. officinalis* harvested in Algeria was dominated by neral, geranial and citronellal. This composition was qualitatively the same that the oils from Serbia (Dukic et al., 2004), Slovak (Holla et al., 1997), Egypt (Shalaby El-Gengaihi and Khattab, 1995), France (Carnat et al., 1998) and Iran (Sadraei et al., 2003); as seen in Table 4. However, limonene was the major component in the samples from Scotland (Damien et al., 2000) (57.5 %), neral was found with only (4.3 %) and geranial was completely absent. Basta et al. (2005) reported that caryophyllene oxide (12.6 %) and  $\beta$ -pinene (18.2 %) were also the most abundant constituents in the oil of *M. officinalis* from Greece but neral and geranial were not detected in the oil. Oils from Cuba (Pino et al., 1999) and Brazil (Da Silva et al., 2005) were dominated by neral (29.9 % and 39.3 %) and geranial (41.0 % and 47.3 %) respectively. A low content (0.2 %) of citronellal was found in leaves of Cuba (Pino et al., 1999) and it is not detected in oil from Brazil (Da Silva et al., 2005). A typical composition from Turkey (Allahverdiyev et al., 2004) is characterized by the occurrence of  $\beta$ -carophyllene (14.2 %), which is drastically different from Algerian oil. Minor compounds were punctually reported. Geraniol in Scotland (5.73 %) (Damien et al., 2000), in Egypt (4.2 %) (Shalaby El-Gengaihi and Khattab, 1995), and in Serbia (3.4 %) (Dukic et al., 2004), against (0.6 %) of the oil from Algeria. Geranyl acetate was present at 5.9 % in Slovak (Holla et al., 1997) and 7.1 % in Iran (Sadraei et al., 2003). Otherwise, several sesquiterpenes have been reported at appreciable content like  $\beta$ -carophyllene 4.9 % in Egypt (Shalaby El-Gengaihi and Khattab, 1995), 4.6 % in Serbia (Dukic et al., 2004), 4.2 % in Slovak (Holla et al., 1997), 4.9 % in Iran (Sadraei et al., 2003), 2.4 % in France (Carnat et al.,

**Table 2:** Results of the antimicrobial activity tests (diameter of the inhibition zones in mm) of the essential oil of *Melissa officinalis* by the paper disk method

Indicator microorganisms	Essential oil (35 µg/ disk)	Essential oil controls (35 µg/disk)			Reference Standards		
		S1	S2	S3	Ampi-cillin	Nal. Ac.	Nysta-tin
<b>Gram-positive bacteria</b>							
<i>Staphylococcus aureus</i>	18	20	18	16.5	21	-	-
<i>Bacillus subtilis</i>	17	17	17	16.5	46	-	-
<i>Listeria monocytogenes</i>	17	17	16	16	30	-	-
<b>Gram-negative bacteria</b>							
<i>Pseudeumonas aeruginosa</i>	19	30	31	31	-	29	-
<i>Escherichia coli</i>	20	20	19	18.5	-	30	-
<i>Klebsiella pneumoniae</i>	21	15.5	15	14.5	-	18	-
<i>Salmonella enterica</i>	14	10	10.5	9	-	19	-
<b>Yeasts</b>							
<i>Candida albicans</i>	36	32.5	35	33	-	-	18
<i>Saccharomyces cerevisiae</i>	38	38	36	36.5	-	-	29
<b>Fungi</b>							
<i>Fusarium oxysporum albedinis</i>	38	30	30	30	-	-	20
<i>Fusarium oxysporum lini</i>	34	25	23.5	21	-	-	24
<i>Mucor ramannianus</i>	39	29	31.5	30	-	-	31

S1: Standard 1 (Citral); S 2: Standard 2 (Citronellal); S3: Standard 3 (Caryophyllene oxide); Nal. Ac : Nalidixic acid; -: absence of inhibition zone detected. The data represent mean values of triplicate determinations. Values include the diameter of disk (6 mm).

**Table 3:** Antimicrobial minimal inhibitory concentrations of *Melissa officinalis* essential oil

Test microorganisms	Essential oil (µL/mL)
<b>Gram-positive bacteria</b>	
<i>Staphylococcus aureus</i>	3
<i>Bacillus subtilis</i>	2
<i>Listeria monocytogenes</i>	2
<b>Gram-negative bacteria</b>	
<i>Pseudeumonas aeruginosa</i>	2
<i>Escherichia coli</i>	2
<i>Klebsiella pneumoniae</i>	3
<i>Salmonella enterica</i>	5
<b>Yeasts</b>	
<i>Candida albicans</i>	3
<i>Saccharomyces cerevisiae</i>	2
<b>Fungi</b>	
<i>Fusarium oxysporum albedinis</i>	2
<i>Fusarium oxysporum lini</i>	1
<i>Mucor ramannianus</i>	1

1998) against 1.3 % in our oil. An oxygenated sesquiterpene, caryophyllene oxide was identified at 10.0 % in Egypt (Shalaby El-Gengaihi and Khattab, 1995), 8.35 % in Slovak (Holla et al., 1997), 5.3 % in Cuba (Pino et al., 1999), 2.7 % in Iran (Sadraei et al., 2003), 1.7 % in Serbia (Dukic et al., 2004) and 1.3 % in Algerian oil. Similar results were obtained with essential oil of *M. officinalis* (Sari and Ceylan, 2002; Dawson et al., 1988; Shalaby El-Gengaihi and Khattab, 1995; Holla et al., 2000; Adzet et al., 1992). The most dominant constituent obtained was citral (geranial and neral).

The essential oil antibacterial activity of *M. officinalis* was reported in some papers (Dukic et al., 2004; Mencheriniet al., 2007; Romeo et al., 2008; Tullio et al. 2007). However, this is the first study of antifungal activity of *M. officinalis* oil against the two *Fusaria* species.

**Table 4:** Main constituents of chemical composition of *Melissa officinalis* of various origins

Constituents	Various origins										
	This Work 2014	Serbia 2004	Slovak 1997	Cuba 1999	Egypt 1995	France 1998	Brazil 2005	Scotland 1995	Iran 2003	Turkey 2004	Greece 2005
$\beta$ -pinene	-	-	-	-	-	-	-	-	-	-	18.2
Limonene	-	2.2	0.1	-	0.7	-	-	57.5	-	-	tr
Linalool	0.3	0.5	0.08	0.6	0.2	0.6	0.8	0.6	0.9	1.3	tr
Citronellal	6.3	13.7	11.3	0.2	13.3	39.5	-	24.9	12.9	2.9	-
Neral	30.2	16.4	22.2	29.9	19.7	20.4	39.3	4.3	24.5	5.8	-
Geraniol	0.6	3.4	-	-	4.2	0.2	-	5.7	0.7	0.4	-
Geranial	44.2	23.4	33.6	41.0	26.8	27.8	47.3	-	35.5	6.6	-
Geranyl acetate	-	0.8	5.9	4.4	1.8	0.6	1.5	-	7.1	-	-
$\beta$ - Caryophyllene	1.3	4.6	4.2	-	4.9	2.4	0.9	-	4.9	14.2	15.3
Caryophyllene oxide	1.3	1.7	8.3	5.3	10.0	-	1.2	-	2.7	-	12.6

## CONCLUSION

The essential oil composition of *M. officinalis* from Algeria was characterized by its high content of monoterpenoids with citral being major (87.2 %). The present study gives a better insight on the volatiles contained in leaves of *M. officinalis* which grows in Algeria and shows similitude and differences with composition oils from different countries in the world. The essential oil of *M. officinalis* revealed strong antimicrobial activity with large inhibition zones and small MIC values against all microorganisms tested. The essential oil represents a complex mixture of different chemical components. This antimicrobial activity of total essential oil cannot be reduced to major components. Other components can contribute to this activity. The present results also demonstrated that *M. officinalis* essential oil can be used in pharmaceuticals and natural therapies of infectious diseases in humans and plants, as well as in food preservation.

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## Declaration of interest

The authors report no conflict of interest. The authors are responsible for the content and writing of the paper.

## REFERENCES

- Adams RP. Identification of essential oil components by gas chromatography/mass spectrometry. Carol Stream, IL: Allured Publishing, 2007.
- Adinee J, Piri K, Karami O. Essential oil component in flower of Lemon Balm (*Melissa officinalis*). Am J Biochem Biotechnol 2008;4:277-8.
- Adjorjan B, Buchbauer G. Biological properties of essential oils: an updated review. Flav Fragr J 2010;25: 407-26.



- Adzet T, Ponz R, Wolf E, Schulte E. Content and composition of *M. officinalis* oil in relation to leaf position and harvest time. *Planta Med* 1992;58:562-4.
- Allahverdiyev A, Duran N, Ozguven M, Koltas S. Antiviral activity of the volatile oils of *Melissa officinalis* L. against Herpes simplex virus type-2. *Phyto-medicine* 2004;11:657-61.
- Basta A, Tzakou O, Couladi M. Composition of the leaves essential oil of *Melissa officinalis* from Greece. *Flav Fragr J* 2005;20:642-4.
- Bauer AW, Kirby WMM, Sherris JC, Turk M. Antibiotic susceptibility testing by a standardized disc method. *Am J Clin Pathol* 1966;45:493-6.
- Beloued A. *Plantes médicinales d'Algérie* (p 134). Alger: Office des Publications Universitaires, 2009.
- Birdane YO, Buyukokuroglu ME, Birdane FM, Cemek M, Yavuz H. Anti-inflammatory and antinociceptive effects of *Melissa Officinalis* L. in rodents. *Rev Méd Vét* 2007;158:75-81.
- Carnat AP, Carnat A, Fraise D, Lamaison JL. The aromatic and polyphenolic composition of lemon balm (*Melissa officinalis* L. Subsp. *officinalis*) tea. *Pharma Acta Helv* 1998;72:301-5.
- Carvalho de Sousa A, Sales Alviano D, Fitzgerald Blank A, Barreto Alves P, Sales Alviano C, Rocha Gattass C. *Melissa officinalis* L. essential oil: antitumoral and antioxidant activities. *J Pharm Pharmacol* 2004;56:677-81.
- Chung MJ, Cho SY, Bhuiyan MJH, Kim KH, Lee SJ. Anti-diabetic effects of lemon balm (*Melissa officinalis*) essential oil on glucose and lipid regulating enzymes in type 2 diabetic mice. *Brit J Nutr* 2010;104:180-8.
- Constantine DS. Extraction, separation and detection methods for phenolic acids and flavonoids. *J Sep Sci* 2007;30:3268-95.
- Damien Dorman HJ, Surai P, Deans SG. In vitro antioxidant activity of plant essential oils and phytoconstituents. *J Essent Oil Res* 2000;12:241-8.
- Da Silva SS, Salgueiro Lage ACL, Da Silva San Gil RA, De Almeida Azevedo D, Esquibel MAJ. Essential oil composition of *Melissa officinalis* L. In vitro produced under the influence of growth regulators. *Braz Chem Soc* 2005;16:1387-90.
- Dawson BSW, Franich RA, Meder R. Essential oil of *Melissa officinalis* L. subsp. *altissima* (Sibth. et Smith) Arcang. *Flav Fragr J* 1988;3:167-70.
- Dukic NM, Bozin B, Sokovic M, Simin N. Antimicrobial and antioxidant activities of (Lamiaceae) essential oil. *J Agric Food Chem* 2004;52:2485-9.
- Holla M, Svajdlenka E, Tekel J, Vaverkova S, Havranek E. Composition of the essential oil from *Melissa officinalis* L. cultivated in Slovak Republic. *J Essent Oil Res* 1997;9:481-4.
- Hussain, AI, Anwar F, Nigam PS, Saker SD, Moore JE, Rao JR et al. Antimicrobial activity of some Lamiaceae essential oils using resazurin as an indicator of cell growth. *LWT* 2011;44:1199-206.
- Jassim SAA, Naji MA. Novel antiviral agent: a medicinal plant perspective. *J Appl Microbiol* 2003;95:412-27.
- Mencherini T, Picerno P, Scesa C, Aquino R. Triterpene, antioxidant and antimicrobial compounds from *Melissa officinalis*. *J Nat Prod* 2007;70:1889-94.
- Mulkens A, Kapetanidis I. Eugenylglucoside, a new natural phenylpropanoid heteroside from *Melissa officinalis*. *J Nat Prod* 1988;51:496-8.
- Oki T, Tenmyo O, Tomatsu K, Kamai H, Pradimicins AB. New antifungal antibiotics. II. In vitro and in vivo biological activities. *J Antib* 1990;30:334-6.
- Pino JA, Rosado A, Fuentes V. Composition of the essential oil of *Melissa officinalis* L. from Cuba. *J Essent Oil Res* 1999;11:363-4.
- Romeo V, Serena De Luca Piscopo A, Poiana M. Antimicrobial effect of some essential oils. *J Essent Oil Res* 2008;20:373-9.
- Sadraei H, Ghannadi A, Malekshahi K. Relaxant effect of essential oil of *Melissa officinalis* and citral on rat ileum contractions. *Fitoterapia* 2003;74:445-52.
- Sari AO, Ceylan A. Yield characteristics and essential oil composition of Lemon balm (*Melissa officinalis* L.) grown in the aegean region of Turkey. *Turk J Agric For* 2002;26:217-24.
- Shalaby El-Gengaihi AS, Khattab M J. Oil of *Melissa officinalis* L. as affected by storage and herb drying. *J Essent Oil Res* 1995;7:667-9.
- Sharafzadeh S, Khosh-Khui M, Javidnia K. Aromatic profile of leaf and stem of Lemon Balm (*Melissa Officinalis*) grown under greenhouse conditions. *Advin Env Biol* 2007;5:547-50.

Spiridon L, Colceru S, Anghel N, Teaca CA, Bodirlau R, Armatu, A. Antioxidant capacity and total phenolic contents of oregano (*Origanum vulgare*), lavender (*Lavandula angustifolia*) and lemon balm (*Melissa officinalis*) from Romania. Nat Prod Res 2011;25:1657-61.

Tagashira M, Ohtake Y. A New antioxidative 1, 3-benzodioxole from *Melissa officinalis*. Planta Med 1998;64:555-8.

Toth I, Mrlanova J, Tekelova D, Korenova M. Rosmarinic acid - an important phenolic active composition of lemon balm (*Melissa officinalis* L.). Acta Facult Pharm 2003;139-46.

Tullio V, Nostro A, Mandras N, Dugo P, Banche G, Cannatelli MA et al. Antifungal activity of essential oils against filamentous fungi determined by broth microdilution. J Appl Microbiol 2007;102:1544-50.

Vitullo M, Ripabelli I, Fanelli I, Tamburro M, Delfino S, Sammarco L. Microbiological and toxicological quality of dried herbs. Lett Appl Microbiol 2011;52: 573-80.

Ziakova A, Brandsteterova E. Validation of HPLC determination of phenolic acids present in some Lamiaceae family plants. J Liquid Chromatogr 2003;26: 443-53.