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Melissa officinalis L. Essential Oil: Chemical Composition, Antioxidant, Antibacterial and Antifungal Activities- *in vitro* Study

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The present investigated chemical composition of *Melissa officinalis* L. essential oil (MOEO) extracted by hydrodistillation. The MOEO was analyzed by gas chromatography-mass spectrometry (GC-MS), revealing the presence of thirty compounds, representing 98.46% of the oil

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constituents. The predominant components were 1,8-cineole (39.80%) followed by citronellol (16.66%), geraniol (12.25%), myrcene (5.85%) and geranial (5.45%). The antioxidant potential of MOEO has been summarized using DPPH test (IC_{50}), superoxide anion ($O_2^{\cdot-}$) scavenging activity (IC_{50}), β-carotene (IC_{50}) and reducing power (FRAP) (EC_{50}). Results demonstrate strong scavenging superoxide anion capacity and moderate to weaker activity against the other assays. Potent inhibitory effect has been observed towards *Micrococcus luteus* and *Bacillus cereus* as well as the *Candida albicans* ATCC 90028, *C. tropicalis* (Strain 1) and *C. albicans* (Strain 8). Our work provides a view for the further studies on the antioxidant and antimicrobial of the MOEO and its main components.

Keywords: Melissa officinalis L; essential oil; GS-MS; antioxidant; antibacterial; antifungal.

1. INTRODUCTION

Essential oils are a complex matrix composed of various volatile compounds which have been recognized for a long time as a powerful reservoir of therapeutic and pharmacological effect with health and nutritional benefits [1-3]. They have been explored as new alternatives in the development of cosmetics and pharmaceuticals products mainly due to their antioxidant and antimicrobial properties. Those properties are mainly associated with their own chemical composition, which is determined by pedoclimatic conditions and plant genotype [4,5]. The high and spread invasive of bacterial and fungal infections, as well as the increasing of drug resistance, accelerate the need for new antioxidants and antimicrobials to overcome infections disease [6-8]. Those from natural product remains the most requested due to their low side effects [9-14].

Melissa officinalis L. (*M. officinalis*) belonging to Lamiaceae family (mint family) is a perennial, subshrub, endemic, herbaceous medicinal plant native to Southern Europe and the Mediterranean region as well as in Central Asia, Serbia, America, and Africa [15-17]. The germination of the plant accrued naturally in sandy and scrubby areas with matured stage has an average height in the range 70–150 cm [18,19]. Traditionally this plant has been explored for its large therapeutic effect to cure various disease, including cardiac, headaches, carminative flatulence, antispasmodic in digestion, colic, nausea, hypersensitivities, amenorrhea, nervousness, anemia, vertigo, syncope, malaise, asthma, rheumatism, bronchitis, failure, arrhythmias, insomnia, epilepsy, depression, psychosis, hysteria, ulcers and wounds [20,21]. Previously studies suggested that the high pharmacological capacity of this plant is mainly related to its richness in secondary metabolites such as flavonoids, phenolic acid, and terpenes [22]. Other secondary metabolites such as eugenol, octinol, octin, octinone, citral, hexenol, and haramin from *M. officinalis* essential oil contribute to its powerful activity [23]. This essential oil of this plant has long been used due to its antioxidant, antifungal and antimicrobial properties [24-26]. The *M. officinalis* essential oil has been demonstrated for its high effect against four bacterial nosocomial infections: *Pseudomonas aeruginosa, Klebsiella pneumonia, Staphylococcus aureus* and *Citrobacter koseri* [16]. Also, the high antimicrobial activity against microorganisms, mainly five human pathogenic bacteria, one yeast, *Candida albicans*, and two phytopathogenic fungi has been reported [23]. Moreover, the essential oil obtained by microdistillation of aerial parts of *M. officinalis* (Bulgarian origin) showed potent antioxidant activity. In another study, the low antioxidant properties of EO of *M. officinalis* was mainly explained by attributed to the low contents of volatile phenolic compounds such as camphor and carvacrol [23].

The aim of the present study was to evaluate the chemical composition, antioxidant, antibacterial and antifungal properties of *M. officinalis* essential oil.

2. MATERIALS AND METHODS

2.1 Plant Material and Essential Oil Isolation

Melissa officinalis L. plants were harvested from a private nursery for aromatic and medicinal plants in the region of Sidi Bouzid (center of Tunisia) and identified according to the flora of Tunisia. Aerial parts were separated and dried at room temperature for about ten days. Once dried, the plant material was extracted for EOs. An amount of 200 g of aerial part was transferred

to hydro-distillation for 3 hours with 1000 mL distilled water using a Clevenger-type apparatus. The distilled EO was dried over anhydrous sodium sulfate, filtered, and stored at 4°C. The yield was calculated based on the dried weight of the sample as follows: Yield (g/100 g) = (W1 x 100)/W2 where W1 is the weight of the EO and W2 is the weight of the dried plant powder.

2.2 Gas Chromatography Mass Spectrometry Analyses of the Essential Oil

The chemical composition of essential oil has been identified using a GC–MS apparatus HP 5890-series (Agilent Technologies, Santa Clara, United States), as described by Hajaloui et al. [1]. The identification of the components was performed by comparison of their retention times with those of pure authentic samples and by mean of their retention indices relative to the series of *n*-hydrocarbons. GC/EIMS analyses were performed with the Varian CP-3800 gas-chromatograph. The identification of the constituents was based on comparison to retention times with those of authentic samples, comparing their linear retention indices relative to the series of *n*-hydrocarbons, components of known oils and MS literature data [27]. Moreover, the molecular weights of all the identified substances were confirmed by GC/CIMS, using methanol as *CI* ionizing.

2.3 Antibacterial Activity

The antibacterial activity was screened based on the same protocol as done by Hajaloui et al. [1]. bacterial strains tested in this study belonged to 10 references, which were presented in Table 2. The bacterial species consisted of 6 Gram-positive and 4 Gram-negative bacterial strains. The diameter of inhibition zone was measured with 1 mm flat rule, and diameters were interpreted according to the Committee of the French Society of the Antibiogram [28].

2.4 Screening for Antifungal Activity

The human pathogenic yeast used in this study was isolated from patients suffering from candidiasis. The strains tested belong to 4 references strains and 26 isolates: *Candida krusei* (n = 1), *Candida tropicalis* (n = 2), *Candida glabrata* (n = 4), *Candida parapsilosis* (n = 1), *Candida sake* (n = 2), *Candida kefyr* (n = 3), *Candida holmii* (n=2) and *Candida albicans* (n = 11). For screening the antifungal activity of *Melissa officinalis* essential oil, the agar-disc diffusion method was described by [29].

2.5 Antioxidant Activity

2.5.1 Scavenging ability on DPPH radical

The DPPH· quenching ability of the EO was measured according to the method cited by [30]. The ability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH}\cdot \text{ scavenging effect (\%)} = [(A_0-A_1)/A_0]*100 \quad (1)$$

Where A_0 refers to the absorbance of the control at 30 min, and A_1 to the absorbance of the sample at 30 min. All samples were analyzed in triplicate.

2.5.2 Superoxide anion radical-scavenging activity

Superoxide anion scavenging activity was assessed using the method cited by [1] with slight modification. Absorbance was read at 560 nm against a blank. Antioxidant activity was evaluated based on IC_{50} values. The inhibition percentage of superoxide anion generation was calculated using the following formula:

$$\text{Superoxide quenching (\%)} = [(A_0 - A_1) \times 100]/A_0$$

Where, A_0 and A_1 had the same references presented in Eq. (1).

2.5.3 Reducing power

The ability of the EO to reduce Fe^{3+} was assayed using the method described by [31]. The EC_{50} value (µg/mL) is the effective concentration at which absorbance was 0.5 for reducing power. BHT and ascorbic acid were used as positive control.

2.5.4 β-Carotene-linoleic acid model system (β-CLAMS)

The β-CLAMS method is based on the discoloration of β-carotene by the peroxides generated during the oxidation of linoleic acid at elevated temperature and was performed based on the protocol done by [32]. The results were expressed as IC_{50} values (µg/mL). All samples were prepared and analyzed in triplicate.

2.6 Statistical Analysis

All experiments were performed in triplicates, and average values were calculated using the SPSS 25.0 statistical package for Windows. Differences in means were calculated using the Duncan's multiple-range tests for means with a 95% confidence interval (P≤0.05).

3. RESULTS AND DISCUSSION

3.1 Essential Oil Composition of MOEO

The isolated MOEO was a yellow color with the yield of 2.75 g/100 g dry weight. The GC-MS analyses led to the identification of 30 different components, representing 98.46 % of the total oil (Table 1).

The main components were1,8-cineole (39.80%) followed by citronellol (16.66%), geraniol (12.25%), The second components in abundance (1.72-5.85%) were myrcene (5.85%), geranial (5.45%), camphor (2.91), γ-terpinene (2.87%), geranyl acetate (2.35%), *β*-elemene (2.11%) and citronellyl acetate (1.72%). The major classes of the MOEO were aliphatic esters (61.02%), oxygenated monoterpenes (83.71%), monoterpene hydrocarbons (12.16%) and sesquiterpene hydrocarbons (2.62%). This composition showed that this MOEO has a richness of compounds known by their important biological activities (Fig. 1)

Table 1. Chemical composition, Kovats indices and percentage composition of *Melissa officinalis* L. essential oil

N°	Compounds	Kovats index (KI) HP-5	%	Identification
1	*α*-Thujene	929	0.17	MS, KI
2	*α*-Pinene	936	1.02	MS, KI
3	Camphene	951	0.06	MS, KI
4	Myrcene	980	5.85	MS, KI
5	Sabinene	992	0.74	MS, KI
6	*α*-Terpinene	1013	0.05	MS, KI
7	1,8-Cineole	1036	39.80	MS, KI
8	*(E)*-β-Ocimene	1054	0.34	MS, KI
9	*γ*-Terpinene	1061	2.87	MS, KI
10	Linalool	1090	0.19	MS, KI
11	*cis*-Sabinene hydrate	1100	1.06	MS, KI
12	β-Thujone	1129	0.09	MS, KI
14	Camphor	1158	2.91	MS, KI
15	*trans*-Pinocarveol	1173	0.17	MS, KI
16	Borneol	1180	0.17	MS, KI
17	Terpinen-4-ol	1193	0.21	MS, KI
18	*α*-Terpineol	1204	0.09	MS, KI
19	*trans*-Piperitol	1216	0.11	MS, KI
20	Citronellol	1232	16.66	MS, KI
21	Neral	1243	0.68	MS, KI
22	Geraniol	1261	12.25	MS, KI
23	Geranial	1274	5.45	MS, KI
24	Carvacrol	1302	0.09	MS, KI
25	Citronellyl acetate	1353	1.72	MS, KI
26	Eugenol	1361	0.77	MS, KI
27	Geranyl acetate	1384	2.35	MS, KI
28	*β*-elemene	1397	2.11	MS, KI
29	*β*-caryophyllene	1426	0.24	MS, KI
30	*trans* α-bergamotene	1438	0.27	MS, KI
Yield (g/100 g dry weight)			2.75	
Monoterpenes Hydrocarbons 12.16				
Oxygenated monoterpenes 83.71				
Sesquiterpene hydrocarbons 2.62				
Total 98.46				

3.2 Antioxidant Activity of MOEO

In this study, four assays have been employed to investigate the antioxidant power this EO, and the results were compared to the commercial standard (BHT) (µg/ml). The results depicted in Fig. 2 shows that MOEO exhibited strongly antioxidant activity towards superoxide anion with IC_{50} = 1.5±0.2 µg/mL, which is 9.66 times higher than the standard, BHT. Also moderate to weaker antioxidant power has been observed against the other three tests with IC_{50}=11.50±0.62µg/mL (DPPH), IC_{50}=75±1µg/mL (*β*-carotene) and EC_{50} = 23±1µg/mL (FRAP), respectively.

3.3 Antibacterial Activity of MOEO

The *in vitro* antibacterial activity MOEO against ten bacterial strains including six Gram positive bacteria and four Gram negative bacteria, has been assessed in comparison with gentamycin as commercial drug, and the data are presented in Table 2. As shown, MOEO affected all microbial strains with inhibition zones (IZ) ranged between 8.00 mm and 23.67 mm and that *Micrococcus luteus* (IZ = 21.33±1.15 mm *vs.*27.67 ± 1.5 mm), and the tow different strains *Bacillus cereus* ATCC 11778 (IZ = 20.67±0.58 mm*vs.*26 ± 1 mm) and Bacillus cereus ATCC 14579 (23.67±0.58*vs.*28 ± 0) are the most sensitive pathogens. Besides that, our results indicate that Gram positive bacteria were less resistant to the MOEO than Gram negative bacteria, due to the difficulty of hydrophobic molecules to enter on the cell wall having an outer layer surrounding it, which allowed them to be less able to affect their growth.

3.4 Antifungal Activity of MOEO

The results of antifungal activity compared to the standard antibiotic, fluconazole, revealed that MOEO inhibited the growth of all tested fungi with the most remarkable inhibitory effect was obtained towards *Candida albicans* ATCC 90028(IZ = 27.33±0.58mm *vs.*18±0mm), *Candida tropicalis* 1(IZ = 15.66±0.57mm *vs.*16 ±0 mm) and *Candida albicans 8* (IZ = 16±1.73 mm *vs.*16 ±0 mm) (Table 3).

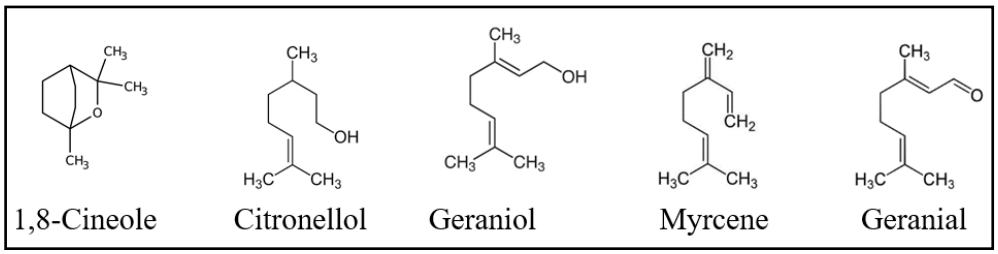

Fig. 1. Structure of the major components of MOEO

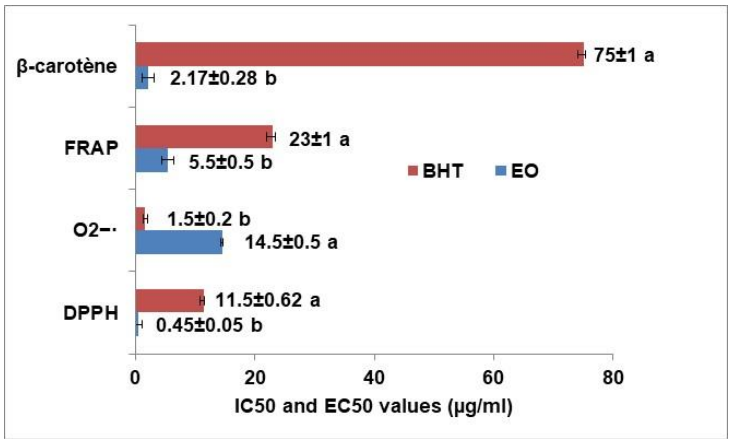

Fig. 2. DPPH test (IC_{50}), Superoxide anion ($O_2^{-\cdot}$) scavenging activity (IC_{50}), reducing power (FRAP) (EC_{50}), and β-carotene of *Melissa officinalis* essential oil (EO) compared to authentic standard (BHT) (µg /ml). Means (three replicates) followed by at least one same letter within a row are not significantly different at *P* < 0.05

Table 2. Zones of growth inhibition (IZ mm±SD), showing the qualitative antibacterial activity of Melissa officinalis essential oil (EO) against human pathogenic bacteria compared to standard antibiotic (Gentamycin)

Strains	IZ (EO)	IZ (Gentamycin)
Gram positive bacteria		
Staphylococcus epidermidis CIP 106510	12 ± 1^{dB}	$21.33 \pm 0.58f^{ghA}$
Staphylococcus aureus ATCC 25923	13.33 ± 0.58^{cB}	32.67 ± 0.58^{aA}
Micrococcus luteus NCIMB 8166	21.33 ± 1.15^{bB}	27.67 ± 1.53^{bA}
Enterococcus feacalis ATCC 29212	14 ± 1^{cB}	26 ± 1^{cA}
Bacillus cereus ATCC 11778	20.67 ± 0.58^{bB}	26 ± 1^{cA}
Bacillus cereus ATCC 14579	23.67 ± 0.58^{aB}	28 ± 0^{bA}
Gram negative bacteria		
Escherichia coli ATCC 35218	10.67^{eB}	22 ± 1^{efgA}
Listeria monocytogenes ATCC19115	13 ± 0^{cdB}	23 ± 0^{deA}
Pseudomonas aeruginosa ATCC 27853	8 ± 0^{fB}	17 ± 1^{iA}
Salmonella typhimurium LT2 DT104	8 ± 0^{fB}	20.33 ± 0.57^{hA}

Table 3. Antifungal activity of *Melissa officinalis* essential oil against several *Candida* species

Strains	Origin	Type of samples	IZ (EO)	IZ (Fluconazole)
Candida albicans	*ATCC 90028*	-	27.33 ± 0.58^{a}	18 ± 0^{b}
Candida glabrata	*ATCC 90030*	-	14 ± 1^{b}	16.33 ± 0.57^{a}
Candida parapsilosis	*ATCC 22019*	-	12.67 ± 0.58^{b}	17.33 ± 0.57^{a}
Candida krusei	*ATCC 6258*	-	12 ± 0^{b}	16 ± 0^{a}
Candida krusei 1	PD-CHU FH	Ear pus	13.33 ± 0.58^{a}	14.33 ± 0.58^{a}
Candida tropicalis 1	PD-CHU FH	Vaginal	15.66 ± 0.57^{a}	14 ± 1^{b}
Candida tropicalis 2	PD-CHU FH	Vaginal	19 ± 1.73^{a}	20 ± 0^{a}
Candida glabrata 1	PD-CHU FH	Oral	20 ± 0^{a}	19.33 ± 0.58^{a}
Candida glabrata 2	PD-CHU FH	CBUE	18.33 ± 1.53^{b}	21.33 ± 0.58^{a}
Candida glabrata 3	PD-CHU FH	Vaginal	11.67 ± 1.15^{b}	14 ± 1^{a}
Candida glabrata 4	PD-CHU FH	Vaginal	11.33 ± 0.58^{b}	13.33 ± 0.58^{a}
Candida Parapsilosis 1	PD-CHU FH	Vaginal	12.66 ± 0.57^{a}	13.33 ± 1.15^{a}
Candida sake 1	MDC	Oral	14 ± 0^{b}	16.33 ± 0.58^{a}
Candida sake 2	MDC	Oral	13.33 ± 0.58^{b}	15 ± 0^{a}
Candida kefyr 1	MDC	Oral	11.66 ± 0.57^{b}	15 ± 0^{a}
Candida kefyr 2	MDC	Oral	13.66 ± 1.15^{b}	17.33 ± 1.15^{a}
Candida kefyr 3	MDC	Oral	17 ± 1^{a}	16.67 ± 0.58^{a}
Candida holmii 1	MDC	Oral	14.83 ± 0.76^{a}	15 ± 0^{a}
Candida holmii 2	MDC	Oral	20 ± 1^{b}	22 ± 0^{a}
Candida albicans 1	MDC	Oral	12.33 ± 0.57^{b}	14.5 ± 0.5^{a}
Candida albicans 2	MDC	Oral	16 ± 1^{a}	17 ± 1^{a}
Candida albicans 3	MDC	Oral	18 ± 1^{b}	20.67 ± 0.58^{a}
Candida albicans 4	MDC	Oral	15.66 ± 0.58^{b}	18.33 ± 0.58^{a}
Candida albicans 5	MDC	Oral	18.67 ± 1.15^{b}	20 ± 0^{a}
Candida albicans 6	MDC	Oral	13 ± 1^{b}	16 ± 0^{a}
Candida albicans 7	MDC	Vaginal	13 ± 1^{b}	15.33 ± 0.58^{a}
Candida albicans 8	MDC	Vaginal	16 ± 1.73^{a}	16 ± 0^{a}
Candida albicans 9	MDC	Vaginal	13 ± 0^{b}	15.33 ± 0.58^{a}
Candida albicans 10	PD-CHU FH	Vaginal	13.33 ± 0.58^{b}	17.33 ± 0.58^{a}
Candida albicans 11	PD-CHU FH	Vaginal	14 ± 1^{a}	14.67 ± 0.58^{a}

SD: Standard deviation; IZ: Inhibition zone diameter (mm) around the discs (6mm) impregnated with 10 µl of essential oil and 20 µg/disc for Amphotericin B (Amp. B); Each value represents the average of 3 repetitions. Means followed by the same letters are not significantly different at p= 0.05 based on Duncan's multiple range test. Small letters (a and b) are used to compare means between IZ EO and IZ Gentamycin for the same strain; PD-CHU FH: Parasitology Department-CHU Farhat Hached; MDC: Monastir Dental Clinic; CBUE: Cytobacterioligical Urine Exam

Mechanistically, EOs penetrate into bacterial cell *via* the membrane inducing a loss of ions, reduction of membrane potential, destruction of proton pump, disruption of bacterial enzyme systems and finally, lysis of the cells [33]. We outlined also that the antimicrobial activity of EOs cannot be easily ascribed to a specific compound, but it depends essentially on the synergistic or antagonistic effects of different compounds in EOs cause antimicrobial activity. EOs composition comprises more than one main compounds with a high concentration that estimate their biological potential. In our study, the biological activity MOEO was significantly related the presence of high level of oxygenated monoterpenes with the main components this oil, are 1,8-cineole (39.80%) followed by citronellol (16.66%), geraniol (12.25%). 1,8-Cineole as the most active compound, plays an important role as adjuvant for antimicrobial purposes since it increases antimicrobial activities when it was combined together with other chemical antibacterial agents were such as mupirocin [34] and chlorhexidine digluconate [35]. Geraniol and citronellol, as two monoterpenes' alcohols produced from combination of two isoprene units have been proven for their efficiency against *Trichophyton rubrum* involves inhibition of ergosterol biosynthesis [36].

4. CONCLUSION

This study demonstrated that MOEO has a significant antioxidant and antimicrobial properties. The EO explained a significant antioxidant capacity better than that obtained by synthetic antioxidant (BHT). These potentials are closely related to the chemical composition showed a wealth of compound interest such as 1,8-Cineole, citronellol geraniol, myrcene and geranial. The richness in oxygenated monoterpenes makes this EO widely used in various applications. In fact, MOEO represents a source of important bioactive compounds with antimicrobial properties which merit further study for possible application in the food industry, where they may be considered as natural preservatives to replace the synthetic ones which consumers are increasingly distrustful. The important actions of the EO against a wide range of strains known for their pathogenicity, let us to suggest that *M. officinalis* can provide an important source of antiseptic agents which can be deserved to be used in pharmaceutical and cosmetic industries.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

NOTE

The study highlights the efficacy of "traditional medicine" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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