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CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF ESSENTIAL OIL FROM *Melissa officinalis* leaves

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ABSTRACT

This study was designed to determine chemical composition and antibacterial activity of essential oil from *Melissa officinalis* leaves. The essential oil was obtained by hydro-distillation and analyzed by gas chromatography-mass spectrometry (GC-MS). Antibacterial activity was evaluated by micro-dilution and disk-diffusion methods. The GC-MS analysis of the essential oil revealed 48 compounds in which D-Limonene, Cyclohexanone, 2-Methyl-5-isopropenyl-2-cyclohexenone,Geraniol and 2-Cyclohexen-1-one, 2-methyl-5-(1-methylethenyl)-, (R)- (CAS) and α-Terpinolene, β-Pinene were the main compounds. Results of disk diffusion method showed inhibition zones of 25.88, 21.33, 16.44 and 12.33 mm respectively against *B. cereus, S. aureus, E. coli* and*S. enterica.* Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of *Melissa officinalis* essential oil against the mentioned bacterial species were respectively 1.04, 3.64, 4.42 and 25 and 1.3, 4.68, 8.33, 25mg/ml. Our findings show significant antibacterial activity for this herbal essential oil which suggests its capacity as a natural food preservative against food-born pathogens.

Keywords: M*elissa officinalis*, GC/MS, Antibacterial activity, D-Limonene, Cyclohexanone.

INTRODUCTION

Spoilage of foods due to the presence of bacterial and fungal infection has been a major concern for decades and causes a considerable loss worldwide (Negi*et al*.2004) and also, the increasing incidence of food-borne diseases, coupled with the resultant social and economic implications, means that there is a constant striving to produce safer food and to develop new natural antimicrobial agents. There is, therefore, still a need for new methods of reducing or eliminating food spoilage and food-borne pathogens, possibly in combination with the existing approaches (K.Bajpai *et al*.2007). The demand for non-toxic, natural preservatives has been rising with increased awareness and reports of ill effects of synthetic chemicals present in food. Furthermore, emergence of food-borne pathogens has lately become a major public health concern (Negi *et al*.2004) and the dramatic increase of infectious diseases, especially those caused by microbial contamination of foods, has become, particularly in underdeveloped countries, an urgent priority. Accordingly, there is a need to develop alternative antimicrobial drugs for their treatment. The use of local medicinal plants for possible antimicrobial and antifungal applications represents a serious promise to satisfy this need (Zarai *et al*.2012). Plant secondary metabolites, such as essential oils and plant extracts, are studied for their antimicrobial activities and most essential oils derived from plants are known to possess antibacterial, insecticidal, antifungal, acaricidal and cytotoxic activities (K.Bajpai *et al*.2007).

Essential oils obtained from aromatic plants have recently gained popularity and scientific interest (Zarai*et al*.2012). Essential oils are components obtained from different plant parts (Gherraf *et al.*2013). Mostly, plant derived essential oils consist of chemical components such as terpenoids including mono-terpenes, sesquiterpenes and their oxygenated derivatives. These compounds have the ability to easily diffuse across cell membrane to induce biological reactions (Naveed*etal.*2013). Essential oils and their main components are used in pharmaceutical, cosmetic and food industries (Saei-dehkordi *et al.*2013), agricultural, food industries (Zellagui *et al*.2012), perfumery manufacturing. These compounds possess a wide spectrum of pharmacological activities (Zarai *et al*.2012).

M. officinalis from Lamiaceae family, with other common names like bee balm, garden balm, melissa, melissengeist, is a perennial herbaceous plant which grows vastly from the central and southern Europe to Iran and central Asia. It is also cultivated worldwide for its edible properties (Ghayoor *et al* 2010; Chen XK *et al* 2006). *Melissa officinalis* L. 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, 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).

The aim of the present study was to investigate the chemical composition of essential oil of *Melissa officinalis* and antibacterial effects of essential oil of this plant.

MATERIAL AND METHODS

Chemicals and Plant materials

Gentamicin (Sinadaroo, Iran), Dimethyl Sulfoxide (DMSO), Muller Hinton (MH) Agar and MH Broth (Merck, Germany) were purchased. The leaves of *Melissa officinalis* was provided from pharmaceutical store at Mashad-Iran during April, 2015. The plant was identified by the vice chancellor for research and technology, Ferdowsi University of Mashhad (Iran).

Essential oil extraction

For the isolation of the essential oil, the hydro-distillation method with the use of a Clevenger apparatus was used. The flowers were carefully cleaned and distilled separately for 3 h. Oil samples were over anhydrous sodium sulfate and stored in sealed vial at low temperature before analysis.

Gas chromatography/mass spectroscopy

The chemical composition of the essential oil was analyzed using GC-MS technique. The chemical composition of the essential oil was analyzed using GC-MS technique. The mass spectrometer was Agilent 6890 N GC/5973MSD-SCAN (Agilent Technologies, Palo Alto, CA, USA) in the electron impact (EI) ionization mode (70ev) and HP- 5MS (bonded and cross-linked 5% phenyl-methylpolysiloxane,30 mm-0.25 mm, coating thickness 0.25 mm) capillary column (Restek, Bellefonte, PA). Injector and detector temperatures were set at 220°C. The oven temperature was held at 50°C for 30 min, then programmed to 240°C at rate of 3°C/min. Helium (99.99%) was the carrier gas at a flow rate of 1 ml/min. Diluted samples (1/100 in hexane, v/v) of 1.0 were injected manually (Ahmadzadeh *et al*. 2014).

Organisms and inoculation conditions

The essential oil of *Melissa officinalis* was individually tested against 4 bacteria strains including *Staphylococcus aureus* (PTCC 1431*),Bacillus cereus* (PTCC 1015), *Salmonella enterica* (PTCC 1709) and *Escherichia Coli* (PTCC 1399) which were obtained from Persian Type Culture Collection, Iranian Research Organization for Science and Technology (PTCC, Iran). Bacterial strains were cultured overnight at 37ºC in MH agar (Yesil Celiktas *et al*. 2007).

Antimicrobial assay

The antibacterial activity of the essential oil and fractions were determined by disk diffusion and micro-dilution methods. All tests were performed in three replicate.

Disk-diffusion method

MHA was poured in petri dishes and surface of cultures was radiated by ultraviolet under the microbial hood that ensures the sterilized condition. Then 100 µL of microbial suspension was poured on the surface of culture and stroke lightly by sterile swab in all over the medium. Then paper disks which were stained by 15 µL essential oil were placed on the surface of medium, after that inoculated plates were incubated in an inverted position at 30Ċ for 24 hrs. The zones of inhibition were recorded by ruler. Finally, the diameter of zones of inhibition compared with positive (Gentamicin antibiotic) and negative (DMSO) controls (koochak *et al*. 2010).

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) Tests

MIC was determined using micro-dilution method according to the protocol of Sahin *et al*.2004. The 96-well plates were prepared by dispensing into each well by 95 µL MHB and 5 µL of the inoculum standardized at 1.5×10^6 CFU/ml by adjusting the optical density to 0.1 at 600 nm by Shimadzu UV-120-01 spectrophotometer (Kuete *et al*.2011). One-hundred microliters of the essential oil was initially prepared at a concentration of 100 mg/ml added into the first well, followed by two-fold dilution until the 8th well. The wells of column 9 were filled with 195 µL of MHB and reserved for the bacterial growth control, whereas the 10th column wells were reserved for the control of the broth sterility. The wells of the last column were used as a negative control. The plates were screened visually after incubation at 37Ċ for 24 hrs for broth turbidity. MIC was defined as the lowest concentration of the essential oil at which the microorganism did not demonstrate visible growth. Microorganism growth was indicated by turbidity. MBC was determined by sub-culturing 10 µl of the MIC test solutions on Mueller-Hinton agar (Merck, Germany) plate at 37°C for 24 h. The highest dilution that yielded no bacterial growth was taken as MBC (Elaissi *et al*.2012).

RESULTS

GC-MS analysis of essential oil

The composition of the essential oil from *M. Afficinalis* is shown in Table 1 which indicates 48 different components in the composition.

Table-1. Chemical composition of *M. Officinalis* essential oil.

Compound	R.time	%
α-Pinene	9.446	1.09
Camphene	10.087	0.14
β-Pinene	11.503	1.96
β-Myrcene	12.337	0.96
l-Phellandrene	12.902	0.30
α-Terpinolene	13.526	0.32
D-Limonene	14.855	60.70
ϒ-Terpinene	15.834	0.99
α-Terpinolene	17.274	1.10
Linalool 1,6-Octadien-3-Ol, 3,7-dimethyl-α-Terpinolene	18.032	1.71
Cis-Rose Oxide 2H-Pyran, tetrahydro-4-methyl-2-(2-methyl-1-propenyl)	18.440	0.28
Limonene oxide, Cis-(1R,2S,4R)-1,2-Epoxy-p-menth-8-ene Cis-Limonene Oxide	19.471	0.18
Terpinene 1-Ol 2,6-Dioxa-tricyclo[3.3.2.0(3,7)]dec-9-ene p-Menth-3-en-1-Ol	19.553	0.14
Trans-Limonene Oxide	19.699	0.12
β-Fenchene	20.083	0.17
L-Menthone	20.503	0.51
Cyclohexanone, 5-methyl-2-(1-methylethyl)-, Cis- (CAS)	21.004	0.32
Borneol	21.074	0.23
3-Cyclohexen-1-Ol, 4-methyl-1-(1-methylethyl)- (CAS)	21.634	0.30
αTerpineol	22.455	1.02
Cyclohexanone, 2-methyl-5-(1-methylethenyl)-, trans-Cis-Dihydrocarvone	22.689	1.15
Ethanol, 2-(dodecyloxy)- (CAS)	22.893	0.21
trans-Carveol	24.105	0.32
Cyclohexanone	24.857	7.67
2-Methyl-5-isopropenyl-2-cyclohexenone	25.277	6.22
2-Cyclohexen-1-one, 2-methyl-5-(1-methylethenyl)-, (R)- (CAS)	25.370	2.62
Geraniol	25.859	2.38
Z-Citral	26.425	1.02
6-Octen-1-ol, 3,7-dimethyl-, Formate	26.611	0.94
Geraniol Formate	26.804	0.16
Geraniol Formate	27.789	0.54
1-Octene Cyclopentane, 1,2,3-trimethyl- 1,2,3-Trimethylcyclopentane	28.610	0.16
Dihydrocarvyl acetate	28.914	0.23
2-Cyclohexen-1-one, 3-methyl-6-(1-methylethylidene)- (CAS)	29.421	0.17
Neryl Acetate	30.540	0.19
α-Copaene	30.936	0.17
Naphthalene, 1,2,3,4-tetrahydro-1, 6-dimethyl-4-(1-methylethyl)-, (1S-Cis)	31.344	0.24
2,6-Octadien-1-ol, 3,7-dimethyl-,acetate 2,6-Octadien-1-Ol, 3,7-dimethyl-,(Z)	31.414	0.41
trans-Caryophyllene	32.865	0.93
3,7-Guaiadiene	33.856	0.17
α-Humulene	34.264	0.17
β-Cubebene	35.424	0.12
Valencene	35.931	0.23
β-Bisabolene	36.596	0.12
Naphthalene, 1,2,3,4-tetrahydro-1, 6-dimethyl-4-(1-methylethyl)-, (1S-Cis)	37.167	0.14
(-)-Caryophyllene oxide	39.533	0.16
Guaiol	40.151	0.23
5-Azulenemethanol, 1,2,3,3a,4,5,6, 7-octahydro-α,α,3,8-tetramethyl-, [3S-(3α,3β ,5α)]	42.803	0.37

Disc-diffusion test

These results indicated that the diameters of inhibition zones varied from 12-25mm and 25-30mm for the various concentrations of essential oil and Gentamycin respectively. Among the four bacteria, *B. cereus* was the most sensitive to the essential oil (25 mm). *S.enterica* was the most resistant to the essential oil (12 mm).

Table 2. Disc-diffusion test and inhibition zones (mm) for the essential oil of *M. officinalis.*

Microorganisms	Essential oil	Positive control Gentamicin	Negative control DMSO
Bacillus cereus	25	30	6
Staphylococcus aureus	21	28	6
Escherichia coli	16	28	6
Salmonella enterica	12	25	6

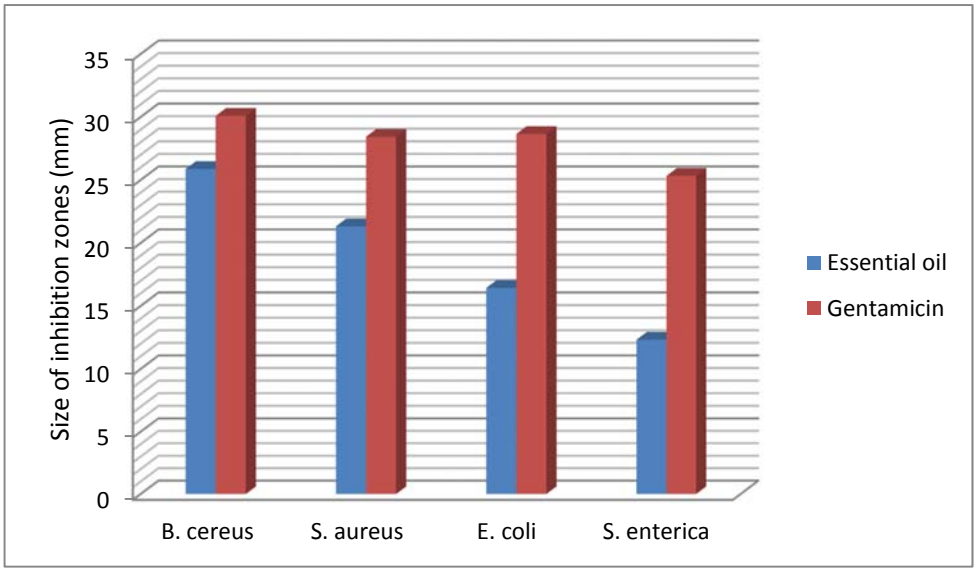

Figure-1. Comparison between inhibition zones of essential oil and Gentamicin (mm).

MIC and MBC data

The MIC values are summarized in Table-3. Results show the essential oil is able to prevent the growth of all the 4 tested bacteria in the range of 1.04-25 mg/ml, with *B. cereus*(1.04 mg/ml)and *S. Enteric* (25 mg/ml)being the most and least sensitive species respectively. So the gram-positive bacteria are more sensitive than the gram-negative bacteria.

The MBC values of the essential oil ranged from 1.3-25 mg/ml, with *B. cereus* (1.3 mg/ml) and *S. Enteric* (25 mg/ml)being the most and least sensitive species, respectively.

Table 3. MIC and MBC values for essential oil of *M.officinalis* (mg/ml)

Microorganism	*B. cereus*	*S. aureus*	*E. coli*	*S. enterica*
MIC	1.04±0.45	3.64±2.38	4.42±3.15	25±21.65
MBC	1.3±0.45	8.33±3.6	4.68±2.7	25±21.65

DISCUSSIONS

Natural products, such as plant extract, either as pure compounds or as standardized extracts, provide unlimited opportunities for control of microbial growth owing to their chemical diversity. Besides antimicrobial, several plants are being used in different areas of human health such as traditional medicine, functional foods, dietary supplements and recombinant protein manufacturing. Phytochemicals, especially flavonoids, polyphenols, anthocyanins and carotenoids, share the major market (Negi, 2012). Flavonoids (derivatives of phenylchromone ring) are a large group of compounds naturally occurring in higher and lower plants. Flavonoids have been shown to be able to affect various biological

functions: capillary permeability, cellular secretory processes involved in the inflammatory response and inhibition of enzymes, receptors and carriers (Sanches *et al*.2005).

The chemical composition of the *M. Officinalis* essential oil has been previously studied and concerned plants from various origins (Sarer and Kokdil, 1991; Sadraei *et al*. 2003). All the investigated leaf oils were characterized by the occurrence of oxygenated monoterpenes as major components. However, several compositions were observed with respect to the contents of the four principal components limonene, citronellal, neral and geranial. The oxygenated compounds of *M. Officinalis* subsp. *Officinalis* oil are reputed to possess sedative, spasmolytic, antimicrobial, antiviral, anti-inflammatory and antioxidative properties (Dukic *et al.* 2004; Damien Dorman *et al*. 2000). Its essential oil is currently used in medicine and pharmacology as an antimicrobial (Mimica *et al*. 2003; Mencherini *et al*.2007), anti-tumor (De Sousa *et al*. 2004), antioxidant (Marongiu *et al*. 2004; Dastmalchi *et al*. 2008), to moderate alzheimer's disease (Khayyal *et al*. 2001), stimulate the immune system (Drozd and Anuszewska, 2003) and possess anti-HIV-1 activity (allahverdiyev *et al*. 2004).

The present study showed D-Limonene (60.70%), Cyclohexanone (7.67%) as the 2 main components. Meftahizade *et al* (2010) reported that the main constituents of the essential oil are Citral, Citronellal, Geraniol, β-Pinene, α-Pinene and β-Caryophyllene, comprising 96% of the oil ingredients. Also Carnat *et al*, reported the chemical composition of essential oil of lemon balm, and found that major components are Citral representing 48, 2% of the essential oil, followed by Citronellal with 39, 7% and Caryophyllene with 2, 37%. In another investigation, the percentage of the main constitutes found by Sarer and Kokdil (1991) were included: α-Pinene (2.86%), β-Pinene (11.37%), Linalool (2.74%), Citronella (5.86%) Borneol (0.62%), Neral (12.22%), and Geraniol (38.13%).

From the microbiological point of view, some studies show that gram-negative bacteria are more resistant to essential oil; others claim the same for gram-positive bacteria. Our findings showed that gram negative bacteria were more resistant than gram positive ones. The internal stability of the bacterial cells depends on the interaction between a series of physiological factors, and the disturbance of this stability, may determine the bacteria's death or the inhibition of its growth. To provide products, which reduce the toxicity risk and at the same time are obtained from a new natural and renewable source becomes a growing and economically viable option. The use of vegetal extracts for antibacterial activity is a consummated fact (Nogueira *et al*. 2014).

CONCLUSIONS

The results presented in this study indicated that essential oil obtained from leaves of *M. Officinalis* possess antibacterial properties. On the basis of the experimental results, it can be postulated that the essential oil of *M. officinalis* has the potent antibacterial properties against some representative food-borne pathogens. Therefore, they could be used as possible food antimicrobial preservative in food industry, but the in vivo studies should be done to evaluate the probable adverse effect on food sensory properties.

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