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Original Article

Melissa officinalis Essential Oil: Chemical Compositions, Antioxidant Potential, Total Phenolic Content and Antimicrobial Activity

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ABSTRACT

Background and Objectives: *Melissa officinalis* belongs to plant Lamiaceae family and is native to Iran as well as other countries. The aim of this study was to identify chemical compositions and antioxidant activity of the *M. officinalis* essential oil (EO). Another aim of this paper was to assess antimicrobial activity of *M. officinalis* EO on growth of clinical and commercial strains causing infection.

Materials and Methods: Chemical compositions of the *M. officinalis* EO were analyzed using gas chromatography-mass spectrometry (GC-MS). The antioxidant potential was assessed using β-carotene/linoleic acid inhibition and 2,2-diphenyl-1-picrylhydrazyl methods. The total phenol content was analyzed using Folin-Ciocalteu method. Antimicrobial activities of the *M. officinalis* EO were assessed using disk diffusion agar, well diffusion agar, micro-well dilution, agar dilution and minimum bactericidal concentration (MBC) methods.

Results: Major compositions of the *M. officinalis* EO included geranyl acetate (27.9 %), citral (14.2%), Z-citral (9.8%) citronellal (8.4%) and citronellol (7.6%). The total phenolic content and antioxidant potential of the *M. officinalis* EO included 51 ±0.50 mg GAE/g and 98 ±0.45 µg/ml, respectively. Minimum inhibitory concentration (MIC) of the *M. officinalis* EO ranged 0.5–4 mg/ml, while the MBC ranged 1–8 mg/ml. A significant correlation was seen between the inhibition zone diameters (IZD) and concentration of the EO. The smallest IZD was reported for various concentrations of the *M. officinalis* EO on *Pseudomonas aeruginosa*.

Conclusions: In general, results showed that the *M. officinalis* EO included greater inhibitory effects on commercial bacterial strains causing infections, compared to those of clinical bacterial strains. The *M. officinalis* EO have the greatest effect on gram-positive bacteria. This compound is an effective free radical scavenger rich in phenolic compounds. Further studies are necessary to investigate toxicity of the *M. officinalis* EO due to its safety for human use.

Keywords: *Melissa officinalis*, Microbial pathogenesis, Inhibition zone diameter, Chemical composition

Introduction

Treatment of microbial infections is mostly based on the use of common therapeutic antibiotics. Since the common therapeutic antibiotics are extensively used, the pathogenic microorganisms have mostly become resistant to these chemicals Furthermore, common therapeutic antibiotics are usually associated with adverse effects including hypersensitivity, immune-suppression and allergic responses. Therefore, screening of the antimicrobial activities of medicinal herbs for the treatment of infections is as natural substitutions of the current chemical antibiotics [1–6]. Essential oils (EO) and secondary metabolites of herbs have many uses in medicine as we as food and hygiene industries [7, 8]. The herbal EO includes various health features including antioxidant and antimicrobial activities [7,9]. Various forms of the activated oxygen, also known as reactive oxygen species (ROS), include free radicals and non-free radical species. [10]. The ROS exert oxidative stress in human body and break its antioxidant defense mechanism; hence, the free radicals assault cell macromolecules resulting in several physiological

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disorders [11]. Relatively, studies have shown that use of chemical preservatives for long terms may result in the prevalence of various cancers. Therefore, use of chemical preservatives has been decreased during the last few decades. Indeed, use of medicinal herbs as preservatives has recently been increased due to their less unwanted side effects [12, 13]. Lemon balm with the scientific name of *Melissa officinalis* belongs to Lamiaceae family of plants and is native to Iran, Central Asia and Europe. In many countries, *M. officinalis* has been cited as a mild sedative, spasmolytic and antimicrobial agent. The *M. officinalis* EO has been reported as an antibacterial, antifungal and antiparasitic compound [14–17]. The main purpose of this study was to investigate chemical compositions and antioxidant activities of the *M. officinalis* EO. Another purpose of the study was to assess antimicrobial activities of the *M. officinalis* EO on growth of clinical and commercial bacterial strains causing infections.

Materials and Methods

Chemicals, reagents and microbial media

Chemicals such as dimethyl sulphoxide (DMSO), butylated hydroxyl toluene (BHT), β-carotene linoleic acid (ß-CL), 2,3,5-triphenyl-tetrazolium chloride (TTC) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) used in this study were purchased from Sigma-Aldrich, USA. Bacteriological peptone broth (BDH Chemicals, UK), tryptone soy broth (TSB) and sodium chloride (East Anglia Chemicals, UK) were also purchased. All solvents used in the study included analytical grades provided by Merck, Germany. All microbial media, Mueller-Hinton agar (MHA) and Mueller-Hinton broth (MHB), were provided by Merck, Germany.

Preparation of *Melissa officinalis* essential oil

After verification of the plants, collected plants were dried in shade at ambient temperature to a constant weight and then powdered using laboratory grinder. The EO was extracted according to the European Pharmacopoeia protocol. Briefly, 20 gr of the powdered *M. officinalis* were exposed to hydro-distillation for 1.5 h using Clevenger. The EO was dried using anhydrous sodium sulfate (Na_2SO_4) and then stored at 4 °C in glass vials until used in biological studies [18]. The extraction yield of *M. officinalis* EO was assessed based on a method by Noshad et al., 2018 [19].

Essential oil analysis (phytochemical and chemical components)

Phytochemical analysis of the *M. officinalis* EO was carried out based on the qualitative methods. Briefly, phenolic (ferric chloride), flavonoids (Shinoda test), saponins (Froth test) and alkaloids (Mayer and Bosshardt) were analyzed [18, 19]. Chemical compositions of the *M. officinalis* EO were analyzed using gas chromatography/mass spectrometry (GC-MS) [21]. In general, 0.1 µl of the *M. officinalis* EO was injected into the GC-MS. Carrier helium gas was used at a flow rate of 1 ml/min and an ionization energy of 70 eV. The individual compositions were identified via analogy of their mass spectra and retention indices (RI) with those of the valid samples and those given in the literature.

Estimation of total phenolic content (TPC)

The Folin-Ciocalteu method was used using TPC. Results were presented as mg of gallic acid (GA)/g of the dried *M. officinalis* EO [22].

Antioxidant potential

The antioxidant potential of the EO was estimated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich, USA) protocol described by Alizadeh Behbahani and Imani Fooladi in 2018 [23] and Alizadeh Behbahani et al. in 2017 [24]. The radical scavenging activity percentage of DPPH was computed using the following equation (1):

$$\text{Scavenging activity (\%)} = (\text{Abs control} - \text{Abs sample} / \text{Abs control}) \times 100$$

Concentration of the *M. officinalis* EO that inhibited 50% of the DPPH radicals was reported as IC_{50} value. Methods of Ozturk et al., (2011) [25] and Noshad et al., (2018) [19] were used for ß-carotene-linoleic acid assay (ß-CL). The average inhibition rate was calculated using the following equation (2):

$$\text{Inhibition rate (\%)} = [(AA_{120} - AC_{120})/ (AC_0 - AC_{120})] \times 100$$

Where, AA_{120}, AC_{120} and AC_0 were the absorbance for the antioxidant potential of samples after 120 min, control samples after 120 min and control samples at the beginning of the experiment (t = 0), respectively.

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Preparation of the bacterial strains

The commercial strains (*Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Staphylococcus epidermidis* ATCC 12228 and *Streptococcus pyogenes* ATCC 19615) and clinically isolated strains (*P. aeruginosa*, *E. coli*, *S. epidermidis* and *S. pyogenes*) causing infections were provided by the Laboratory of Industrial Microbiology, Ferdowsi University of Mashhad (FUM), Mashhad, Iran. Identification of the clinical strains causing infections was carried out through biochemical tests [26].

Suspension preparation

All bacteria were cultivated on MHA under aerobic conditions for 24 h at 37 °C. Freshly cultured commercial strain and clinically isolated strain colonies were suspended in 10 ml of normal saline. Then, bacterial concentrations were adjusted to 1.5×10^8 CFU (colony forming units)/ml based on 0.5 McFarland [27].

Antimicrobial activity assay

Disc diffusion agar (DDA): Antimicrobial activity of the EO was assessed using DDA method [28]. In this method, 0.1 ml of the incubated bacterial (either clinical or commercial) suspension was spread on MHA plates. Concentrations of 0.5, 1, 2, 4 and 8 mg/ml of the EO were prepared in dimethyl sulfoxide (DMSO) solvent. These were sterilized using 0.22-µm syringe microfilters. A paper disc with 6.2 mm in diameter (Padtanteb, Iran) was infused with 20 µl of the *M. officinalis* EO and placed on inoculated MHA plates. The culture media containing either clinical or commercial bacterial strain were incubated at 37 °C for 24 h. Finally, IZD (mm) around the disks was measured using ruler and recorded [13, 21].

Well diffusion agar (WDA): Antimicrobial activity of the EO was assessed using WDA method [29]. In this method, 5 wells were created on the surface of MHA plates using of Pasteur pipettes. A 0.1 ml of the incubated bacterial (either clinical and or commercial) suspension was spread on the culture media. Concentrations of 0.5, 1, 2, 4 and 8 mg/ml of the EO were prepared. Then, 20 µl of each concentration was poured into a well. A well was left as blank. After incubation for 24 h at 37 °C, plates were studied for IZD [30].

Microwell plate dilution assay: The MIC of the *M. officinalis* EO against the commercial strains causing infections and clinically isolated strains was assessed using micro-well dilution assay according to the protocols by the Clinical and Laboratory Standards Institute (CLSI, 2006a, b, c) [31–33]. Final concentration of the *M. officinalis* EO in the 96-well plate ranged 0.5–16 mg/ml. Then, the 96-well plate was incubated at 37 °C for 24 h [34]. After incubation, 20 µl of 5% 2,3,5-triphenyl-tetrazolium chloride was added to each well. The first concentration, at which the red color was not observed, was considered as MIC [35, 36].

Agar dilution method (ADM): The ADM method was used according to the instruction by CLSI; modified by Wiegand et al. (2008) [37] and Noshad et al. (2018) [19].

Minimum bactericidal concentration (MBC): The MBC was assessed using pour plate method [38]. Briefly, 100 µl of the solution from the wells free of red color (the MIC test) were cultured on MHA plates. The first concentration with bacterial growth on MHA was reported as MBC [19, 29].

Statistical analysis

All experiments were carried out in triplicate. The SPSS software (IBM Analytics, USA) was used to analyze data. Data were considered as significant when $P \leq 0.05$.

Results

Chemical composition, phytochemical analysis and antioxidant potential

Analysis of the *M. officinalis* EO extraction showed that 0.1 ml of the EO was obtained from 20 g of the *M. officinalis* with an extraction yield of 0.5% (v/w). Results of the EO composition analysis by GC-MS showed that the total composition constituted nearly 96.7% of the EO. Geranyl acetate with 27.9% was the major compound of the *M. officinalis* EO (Table 1).

Table 1. Chemical compositions of the *Melissa officinalis* essential oil

No.	Compound	%
1	Geranyl acetate	27.9
2	Citral	14.2
3	Z-citral	9.8
4	Citronellal	8.4
5	Citronellol	7.6
6	Geraniol	5.31
7	(Z)-nerol	5.26
8	Germacrene-D	5.20
9	Nerol	3.45
10	Linalool	1.01

Compounds with percentages less than one are not shown in the table

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Other principal components included citral, Z-citral, geraniol, (Z)-nerol, germacrene-D, nerol, linalool, citronellal and citronellol. Results of the total phenolic content and antioxidant activity of the *M. officinalis* EO are shown in Table 2. Results from the identification of *M. officinalis* EO phytochemical analysis revealed that the essential oil contained alkaloids (yellow or brown), flavonoids (red solution) saponins (formation of a stable foam) and phenolics (green-bluish). Total phenolic content and antioxidant potential of the *M. officinalis* EO included51 ±0.50 mg GAE/g and 98 ±0.45 µg/ml, respectively. The ß-carotene/linoleic acid inhibition test for assessing the antioxidant potential of *M. officinalis* EO showed a 25.22% value.

Antimicrobial activity

Antibacterial effects of the *M. officinalis* EO on growth of clinical and commercial strains causing infections were investigated usingDDA method (Table 3). Results showed that the *M. officinalis* EO included the most antimicrobial effect on *S. pyogenes* (commercial strain) at 8 mg/ml. The smallest IZD of various *M. officinalis* EO concentrations belonged to gram-negative bacteria (clinical strains). Results showed no IZD for *P. aeruginosa* (clinical strain) at concentrations of 0.5 and 1 mg/ml. No IZD was seen for *E. coli* (clinical strain) and *P. aeruginosa* (commercial strain) at concentration of 0.5 mg/ml. The IZD was observed at all *M. officinalis* EO concentrations for *S. pyogenes* and *S. epidermidis*. Results demonstrated that the highlighted bacteria were significantly different ($P \leq 0.05$) in susceptibility to various concentrations of the *M. officinalis* EO using DDA method. Pairwise comparison of the effects of *M. officinalis* EO concentrations on the bacteria (commercial and clinical strains) showed no significant differences. As the concentration increased, the IZD increased too. However, as shown in Table 3, no significant differences ($P \leq 0.05$) were seen between the concentrations of 4 and 8 mg/ml in *E. coli* and *S. pyogenes* (clinical strains).

Table 2. Phytochemical analysis, total phenolic contents and antioxidant potentials of the *Melissa officinalis* essential oil

Chemical	Verification method	Observation	Occurrence
Alkaloids	Mayer and Bosshardt	Formation of a yellow or brown color	+
Flavonoids	Shinoda test	Red solution	+
Saponins	Froth test	Formation of a stable foam	+
Phenolics	Ferric chloride	Green-bluish	+++
IC_{50}	-	-	98.00 ±0.45 µg/ml
ß-CL	-	-	25.22%
TPC	-	-	51.00 ±0.50 mg GAE/g
BHT	-	-	11.20 ±0.50 µg/ml
Control			0

+present in small concentrations; ++present in moderately high concentrations; IC_{50}, the half maximal inhibitory concentration; ß –CL, β-carotene linoleic acid; TPC, total phenolic content; BHT, butylated hydroxyl toluene

Table 3. Mean inhibition zone diameters (mm) of *Melissa officinalis* essential oil on clinical and commercial bacterial strains causing infections (DDA)

Concentration / Microorganism	0.5 mg/ml	1 mg/ml	2 mg/ml	4 mg/ml	8 mg/ml
Clinical strain					
P. aeruginosa	-	-	7.30 ±0.50^{a}	9.10 ±0.50^{b}	10.60 ±0.54^{c}
E. coli	-	7.00 ±0.50^{a}	8.60 ±0.52^{b}	10.30 ±0.54^{c}	11.00 ±0.52^{c}
S. epidermidis	7.00 ±0.52^{a}	9.00 ±0.45^{b}	10.60 ±0.50^{c}	12.40 ±0.54^{d}	13.50 ±0.52^{d}
S. pyogenes	7.30 ±0.50^{a}	9.00 ±0.50^{b}	11.00 ±0.50^{c}	12.60 ±0.50^{d}	14.40 ±0.54^{e}
Commercial strain					
P. aeruginosa	-	7.10 ±0.50^{a}	8.40 ±0.45^{b}	10.00 ±0.52^{c}	11.80 ±0.50^{d}
E. coli	6.50 ±0.34^{a}	7.30 ±0.54^{a}	9.00 ±0.50^{b}	10.80 ±0.50^{c}	11.80 ±0.50^{c}
S. epidermidis	7.70 ±0.50^{a}	9.10 ±0.50^{b}	11.20 ±0.45^{c}	13.80 ±0.50^{d}	14.50 ±0.50^{d}
S. pyogenes	8.20 ±0.45^{a}	9.80 ±0.35^{b}	11.90 ±0.50^{c}	14.30 ±0.52^{d}	15.10 ±0.52^{d}

Values are expressed as mean ±standard deviations, $n = 3$; different letters (a, b, c, d and e) in each row show significant difference at $P \leq 0.05$

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Results of the antibacterial effects of *M. officinalis* EO using WDA method are shown in Table 4. These results revealed that the *M. officinalis* EO was effective on gram-positive bacteria (*S. epidermidis* and *S. pyogenes*) as well as gram-negative bacteria (*E. coli*) at all concentrations. However, no IZD was observed for the gram-negative bacteria (*P. aeruginosa*) at 0.5 mg/ml concentration. Furthermore, results showed that the IZD values in WDA method were greater than those in DDM method. In WDA method, the IZD values were greater than those in DDA method due to direct contacts between the antimicrobial agents and the bacteria. A significant correlation was seen between the IZD value and the concentration of the EO. The smallest IZD value belonged to the various concentrations of *M. officinalis* EO on *P. aeruginosa*. Results showed that the *M. officinalis* EO included a greater inhibitory effect on the commercial strains causing infections, compared to that on clinical strains. The largest mean IZD of 15.80 ±0.52 mm at 8 mg/ml concentrations belonged to *S. pyogenes*. Results of MIC, MBC and antibiofilm activities of the *M. officinalis* EO are summarized in Table 5. The MIC of *M. officinalis* EO ranged 0.5–4 mg/ml, while MBC ranged 1–8 mg/ml. The MIC of *M. officinalis* EO included 0.5, 1, 4 and 4 mg/ml for *S. pyogenes*, *S. epidermidis*, *E. coli* and *P. aeruginosa* (commercial strains), respectively. The MBC of *M. officinalis* EO included 1, 1, 4 and 8 mg/ml for *S. pyogenes*, *S. epidermidis*, *E. coli* and *P. aeruginosa* (commercial strains), respectively. The MIC of *M. officinalis* EO included 1, 2, 4 and 4 mg/ml for *S. pyogenes*, *S. epidermidis*, *E. coli* and *P. aeruginosa* (clinical strains), respectively. The MBC of *M. officinalis* EO included 2, 2, 8 and 8 mg/ml for *S. pyogenes*, *S. epidermidis*, *E. coli* and *P. aeruginosa* (clinical strains), respectively.

Table 4. Mean inhibition zone diameters (mm) of the *Melissa officinalis* essential oil on clinical and commercial bacterial strains causing infections (WDA)

Concentration / Microorganism	0.5 mg/ml	1 mg/ml	2 mg/ml	4 mg/ml	8 mg/ml
Clinical strain					
P. aeruginosa	-	6.80 ±0.50^{a}	8.20 ±0.50^{b}	9.70 ±0.50^{c}	11.00 ±0.50^{c}
E. coli	6.70 ±0.50^{a}	7.80 ±0.50^{a}	9.00 ±0.50^{a}	10.80 ±0.52^{b}	12.00 ±0.52^{b}
S. epidermidis	7.60 ±0.50^{a}	9.00 ±0.50^{a}	11.10 ±0.52^{b}	13.20 ±0.50^{c}	14.70 ±0.52^{d}
S. pyogenes	8.20 ± 0.50^{a}	9.80 ±0.50^{b}	12.00 ±0.54^{c}	14.50 ±0.50^{d}	15.10 ±0.50^{d}
Commercial strain					
P. aeruginosa	-	7.20 ±0.50^{a}	8.80 ±0.45^{b}	10.10 ±0.50^{b}	11.20 ±0.54^{b}
E. coli	7.00 ±0.50^{a}	7.90 ±0.54 ^{a}	9.20 ±0.50^{a}	11.00 ±0.50^{b}	12.30 ±0.50^{b}
S. epidermidis	8.00 ±0.52^{a}	9.30 ±0.50^{a}	11.40 ±0.50^{b}	14.00 ±0.52^{c}	15.10 ±0.50^{c}
S. pyogenes	8.60 ±0.45^{a}	10.10 ±0.50^{b}	12.30 ±0.50^{c}	14.90 ±0.52^{d}	15.80 ±0.52^{d}

Values are expressed as mean ± standard deviations, $n = 3$; different letters (a, b, c, d and e) in each row show significant difference at $P \leq 0.05$

Table 5. Minimum inhibitory concentration and minimum bactericidal concentration of the *Melissa officinalis* essential oil on clinical and commercial bacterial strains causing infections

Microorganism	MIC (micro-well dilution) (mg/ml)	MIC (agar dilution) (mg/ml)	MBC (mg/ml)
Clinical strain			
P. aeruginosa	4	≤ 4	8
E. coli	4	≤ 4	8
S. epidermidis	2	≤ 2	2
S. pyogenes	1	≥ 1	2
Commercial strain			
P. aeruginosa	4	> 4	8
E. coli	4	≤ 4	4
S. epidermidis	1	≥ 1	1
S. pyogenes	0.5	≥ 0.5	1

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Discussion

Chemical composition, phytochemical analysis and antioxidant activity

In 2003, Sadraei et al. [16] showed that the *M. officinalis* EO contained 0.1% (v/w) of yellowish EO. Their results demonstrated that 17 constituents identified in *M. officinalis* EO accounted for 98.1% of the total oil composition. In 2004, de Sousa et al. [15] demonstrated that the yield of *M. officinalis* EO included 0.97% (v/w). Pouyanfar et al. (2018) [17] reported that the average *M. officinalis* EO yield included 0.13–0.35% (w/w). During the growth cycles, the environmental agents such as amount of existing water are very important in yield of dried materials and EOs [17, 39]. Mimica-Dukic et al. (2004) [40] studied the *M. officinalis* EO chemical compositions. They showed that the chemical compositions *M. officinalis* EO included monoterpene aldehydes, ketones and sesquiterpene. Adinee et al. (2008) [41] investigated the *M. officinalis* EO chemical compositions. They reported that trans-carveol was dominant within the components. Other principal components included trans-carveol (28.89%), citronellol (25.24%), □-3-carene (5.26%), citronellal (4.9%), geraniol (2.2%), 1-octene-3-ol (2.03%) and spathulenol (2.06%). In 2004, de Sousa et al. [15] reported that the major compositions in 13 EOs included limonene-10-ol, geraniol and caryophyllene oxide. These were reported from various countries previously. Chung et al. (2010) [14] showed 40 compositions in EO of the *Melissa officinalis* leaves. These majorly included monoterpene hydrocarbons, sesquiterpene hydrocarbons and oxygenated monoterpenes. In 2014, Uyanik and Gurbuz [42] demonstrated that citral, caryophyllene oxide and zcitral (in leaves and flowers), caryophyllene oxide, citral and β-caryophyllene (in whole plant) were dominant within the components. Bozovic et al. (2018) [43] reported cis-caryophyllene and its oxide form as the major EO constituents; similar to reports from the current study. Alizadeh Behbahani et al. (2017) showed that the chemical compounds of the EO varied depending upon the herbal variety, growth stage, and collection time and the climatic condition [20]. Previously, Mimica-Dukic et al. (2004) [40] showed thatthe*M. officinalis* EO exhibited a very strong free radical scavenging capacity, reducing the DPPH radical formation (IC_{50} = 7.58 μg/ml) and OH radical generation (IC_{50} = 1.74 μg/ml) in a dose-dependent manner. Researches have demonstrated a direct relationship between the total phenolic content and the antioxidant potential [20, 29].

Antimicrobial activity

Many herb EOs owe their ability to the presence of substances such as alkaloids, tannins, glycosides, flavones, anthraquinones, flavonoids, terpenes, anthocyanins, phenolics and aldehydes. These substances are generally found in multiple parts of the herbs, including leaves, roots, shoots, blossoms, flowers and barks. Many herbs have thus become the origins of major drugs and the pharmaceutical industries have become interested in these herbs as sources of bioactive factors [44–46]. Results of this study plainly revealed that the *M. officinalis* EO antibacterial activities on the growth of clinical and commercial strains causing infections. The effectiveness of the active components in herb EOs results in visible IZD surrounding the antimicrobial paper disks. However, some herbal EOs were unable to exhibit antimicrobial activity on clinical and commercial bacterial strains causing infections. The IZD of the *M. officinalis* EO varied from 10.60 mm at 8 mg/ml concentrations for *P. aeruginosa* to 14.40 mm at 8 mg/ml concentrations for *S. pyogenes*. In general, resistance profile of the clinical and commercial strains causing infections was as follows: *P. aeruginosa* > *E. coli* > *S. epidermidis* > *S. pyogenes*. According to studies, gram-negative bacteria are more resistant to the EO than gram-positive bacteria due to the external cell walls [13, 19, 20]. Results showed that MBC of the *M. officinalis* EO was seen at higher concentrations than MIC. Therefore, this suggested that the antibacterial substances in the EO were bacteriostatic at lower and bactericidal at higher concentrations. In 2004, Mimica-Dukic et al. [40] tested antimicrobial effects of the *M. officinalis* EO on bacterial (*P. aeruginosa*, *E. coli*, *S. aureus*, *S. epidermidis*, *Shigella sonnei*, *Sarcina lutea*, *Micrococcus flavus*, *Bacillus subtilis*, *Salmonella enteritidis* and *S. typhi*) and six fungi (*Trichophyton mentagrophytes*, *T. rubrum*, *T. tonsurans*, *Microsporum canis*, *Epidermophyton floccosum* and *Candida albicans*) strains *in vitro*. Their reported that the most effective antimicrobial was expressed on a multi-resistant

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strain of *S. sonnei*. A significant rate of antifungal effects was exhibited in *Trichophyton* spp. Studies have been carried out on antimicrobial effects of herbal EOs on gram-negative and gram-positive bacteria by Yeganagi et al., (2018) [47], Noshad et al. (2018) [19], Alizadeh Behbahani and Imani Fooladi (2018) [23] and Alizadeh Behbahani et al. (2017, 2018) [21, 28]. In these studies, gram-negative bacteria were more resistant to EOs than gram-positive bacteria; as shown by the findings of the present study.

Conclusions

In this study, *M. officinalis* EO was extracted using Clevenger and hydro distillation technique. A GC-MS analysis of *M. officinalis* EO detected 22 compounds, representing 96.7% of the total oil composition. Major composition of the *M. officinalis* EO included geranyl acetate (27.9 %), citral (14.2%), Z-citral (9.8%) citronellal (8.4%) and citronellol (7.6%). Results showed that the smallest IZD on different *M. officinalis* EO concentrations on *P. aeruginosa*. Moreover, results demonstrated that the *M. officinalis* EO included greater inhibitory effects on commercial strains causing infections, compared to clinical strains. The *M. officinalis* EO included the greatest effects on gram-positive bacteria. Normally, *M. officinalis* EO is rich in phenolic compounds and hence an impressive free radical scavenger. Further studies are necessary to investigate potential toxicities of the extracts from *M. officinalis* EO due to the human safety.

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