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Research Article

ANTIBACTERIAL ACTIVITY OF THE ESSENTIAL OIL OF *MELISSA OFFICINALIS* L. AGAINST SOME IMPORTANT FOOD-BORNE PATHOGENIC AND ANTIBIOTIC RESISTANT BACTERIA

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ABSTRACT

Melissa officinalis L. commonly called Lemon balm, member of Lamiaceae family, is one of the important medicinal plant species. That can be used in different branches of industry such as medicine, perfume, cosmetic and food etc. The aim of this study was to evaluate the antibacterial activity of *M. officinalis* essential oil against some important food-borne pathogenic and antibiotic resistant bacteria such as *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Salmonella enteritidis* and *Klebsiella pnomoniae*. The essential oil obtained by hydro-distillation was investigated in different concentrations (0.31-80 µL) using the disc diffusion method and determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Susceptibility testing to antibiotics was carried out using the disc diffusion method. The results of antibacterial activity showed that 20 µL/disc of the essential oil had the highest inhibition zone diameter against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, and *Klebsiella pnomoniae* (45 mm) and the lowest against *Salmonella enteritidis* (15 mm). Also, the essential oil was the most active showing MIC and MBC against *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pnomoniae* (0.31 µL/mL). The highest MIC and MBC obtained against *Bacillus cereus* (20 µL/mL) and *Salmonella enteritidis* (10 µL/mL). The results of susceptibility testing indicated that selected bacterial strains were resistance to some tested standard antibiotics. Therefore, comparison between the results of antibacterial activity and susceptibility testing demonstrated that *M. officinalis* essential oil can be used instead of some tested antibiotics against the 5 selected bacterial strains.

Keywords: *Melissa Officinalis L., Lemon Balm, Essential Oil, Antibacterial Activity*

INTRODUCTION

Melissa officinalis L. commonly called Lemon balm, member of Lamiaceae family, is a perennial, aromatic herb native to southern Europe and northern Africa, and east as far as the Caucasus and northern Iran.

Due to intense lemon aroma and flavor of leaves, *M. officinalis* is used widely in food and cosmetics. It is considered as an important medicinal plant largely used in traditional medicine, in treatment of Alzheimer's disease, as antioxidant against negative effects of free radicals and an antitumoral agent and it has positive effect on immune system and stress (Bahtiyarca and Coşge, 2006; Stanojevic *et al.*, 2010; Abdellatif *et al.*, 2014).

The essential oil content of *M. officinalis* leaves was significantly affected by harvesting stages. The most essential oil was in before flowering stage (Saeb and Gholamrezaee, 2012).

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 (Abdellatif *et al.*, 2014).

The aim of this study was to evaluate the antibacterial activity of *M. officinalis* essential oil against 5 selected food-borne pathogenic and antibiotic resistant bacteria.

MATERIALS AND METHODS

Isolation of the Essential Oil

Leaves of *M. officinalis* plant were collected from northern Iran. The plant materials were dried in the shade at room temperature. A portion (100 g) of them was chopped and subjected to hydro-distillation for 3 hours using a Clevenger-type apparatus, according to the method recommended by European Pharmacopoeia. The obtained *M. officinalis* essential oil was dried over anhydrous sodium sulphate for 24 h, filtered (pore size 0.22 µm), and then stored at 4°C in sealed brown glass vials until tested.

Preparation of Bacterial Strains

Lyophilized bacteria obtained from Institute Pastour (Tehran, Iran). These strains are: two Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus cereus*), three Gram-negative bacteria (*Escherichia coli, Salmonella enteritidis* and *Klebsiella pnomoniae*). All strains were grown in the tubes containing 0.5 mL of BHI (Brain Heart Infusion) broth incubated at 37°C for 1 h. They were maintained on BHI agar slant at 4°C after incubation at 37°C for 24 h.

Standardization of Inoculums

The strains were followed by streaking on MHA (Muller-Hinton Agar) and incubated at 37°C for 24 h. Isolated bacteria from 24 h culture were suspended into sterile normal saline by sterile loop to compare their turbidity to 0.5 McFarland standard which is approximately 1.5×10^{8} CFU/mL. Also, optic density was read for each tested bacterium at a wavelength of 600 nm= 0.08 to 0.13 by specterophotometer.

Inhibitory Effect via the Disc Diffusion Method

The disc diffusion method was used to determine the antibacterial activity of the essential oil. At first, concentrations of 2.5, 5, 10, 20, 40, 50, 80 and 100% from *M. officinalis* essential oil in DMSO (dimethyl sulfoxide) were prepared. Then 100 µL of inoculum was spread over the surface of pre-dried MHA in the Petri plates. Sterile blank filter paper discs of 6 mm in diameter (Padtan Teb, Iran) were placed in each plate. The discs were wetted with 20 µL of prepared different concentrations. Therefore, amount of the essential oil was 0.5, 1, 2, 4, 8, 10, 16 and 20 µL/disc, respectively. 20 µL/disc of DMSO was used as the negative control. The standard antibiotics, namely, gentamicin (10 µg/disc), erythromycin (15 µg/disc), streptomycin (10 µg/disc), penicillin (10 µg/disc), tetracycline (30 µg/disc), sulfamethoxazole/trimethoprim (1.25 µg/23.75 µg/disc) and trimethoprim (5 µg/disc) were used as the positive controls for the test bacteria. All plates were kept at 4°C for 2 h to stop the bacteria from multiplication. Then they were incubated at 37°C for 24 h. The antibacterial activity was evaluated by measuring the inhibition zone diameter (mm) against the tested bacteria. The experiments were repeated in triplicate and the results are expressed as average values.

Determination of Minimum Inhibitory Concentration (MIC)

The MICs of the essential oil against tested bacterial strains were determined using 12 tubes containing 1 mL MHB (Muller-Hinton Broth). In tubes no. 1 to 9 were poured 0.62, 1.25, 2.5, 5, 10, 20, 40, 80 and 160 µL of 50% *M. officinalis* essential oil in DMSO (v/v), respectively. Therefore, amount of the essential oil was 0.31, 0.62, 1.25, 2.5, 5, 10, 20, 40 and 80 µL/mL, respectively. The tube no. 10 was as negative control containing 0.62 µL of 50% the essential oil. The tubes no. 11 and 12 were as positive controls containing 20 µL of DMSO and without the essential oil or DMSO, respectively. Then 50 µL of inoculum was added to all tubes except tube no. 10. All tubes incubated at 37°C for 24 h. The MIC was defined as the lowest concentration of the essential oil that bacterial strains does not demonstrate visible growth in it.

Determination of Minimum Bactericidal Concentration (MBC)

In this assay, 5 µL from each tube was spread over the surface of plates containing MHA. Then plates were incubated at 37°C for 24 h. The MBC is defined as the lowest concentration of the essential oil that incubated bacterial strains are completely killed in it. Each experiment was repeated three times.

RESULTS AND DISCUSSION

The hydro-distillation of 100 g dried plant materials yielded 0.5 g/100gdw the essential oil. The antibacterial activity of *M. officinalis* essential oil was evaluated using the disc diffusion method and determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against *Staphylococcus aureus* and *Bacillus cereus* (Gram-positive), *Escherichia coli*, *Salmonella enteritidis* and *Klebsiella pnomoniae* (Gram-negative).

The results of the present study are shown in Tables 1, 2, 3 and 4. The results of the disc diffusion method via measuring inhibition zone diameter (mm) in different concentrations of *M. officinalis* essential oil (Table 1) indicated that the essential oil had antibacterial activity against all 5 tested bacterial strains. The *Staphylococcus aureus*, *Bacillus cereus* and *Klebsiella pnomoniae* were more sensitive to the lowest concentration of *M. officinalis* essential oil (0.5 µL/disc) than *Escherichia coli* and *Salmonella enteritidis*. Inhibition zones were seen from concentration of 1 µL/disc of the essential oil for *Escherichia coli* and *Salmonella enteritidis*. In the most concentration of the essential oil (20 µL/disc), diameters of inhibition zones were 45 mm for *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Klebsiella pnomoniae* and 15 mm for *Salmonella enteritidis*. The negative control had no antibacterial activity.

Table 1: Mean inhibition zone diameter (mm) in different concentrations of *M. officinalis* essential oil by the disc diffusion method against selected bacterial strains

	Bacterial strain	**Different concentrations of the essential oil (µL/disc)**								
		0.5	**1**	**2**	**4**	**8**	**10**	**16**	**20**	**CON-**
Gram-positive	*Staphylococcus aureus*	8	10	16	23	45	45	45	45	NA
	Bacillus cereus	13	19	36	45	45	45	45	45	NA
Gram-negative	*Escherichia coli*	NA	9	16	23	41	45	45	45	NA
	Salmonella enteritidis	NA	6.5	6.5	11	12	13	15	15	NA
	Klebsiella pnomoniae	15	20	23	45	45	45	45	45	NA

CON-: negative control, DMSO

NA: no antibacterial activity

The results of susceptibility testing of selected bacterial strains to different standard antibiotics via measuring inhibition zone diameter (mm) by the disc diffusion method (Table 2) indicated that *Staphylococcus aureus* and *Klebsiella pnomoniae* to penicillin (10 µg/disc); *Bacillus cereus* to penicillin (10 µg/disc), sulfamethoxazole/trimethoprim (1.25 µg/23.75 µg/disc) and trimethoprim (5 µg/disc); *Escherichia coli* to erythromycin (15 µg/disc), penicillin (10 µg/disc), sulfamethoxazole/trimethoprim (1.25 µg/23.75 µg/disc) and trimethoprim (5 µg/disc) and *Salmonella enteritidis* to erythromycin (15 µg/disc) and penicillin (10 µg/disc) were resistance.

Comparison between the results of antibacterial activity of *M. officinalis* essential oil (Table 1) and susceptibility testing of selected bacterial strains to different standard antibiotics (Table 2) demonstrated that *M. officinalis* essential oil can be used instead of antibiotics of erythromycin (15 µg/disc), penicillin

(10 µg/disc), sulfamethoxazole/trimethoprim (1.25 µg/23.75 µg/disc) and trimethoprim (5 µg/disc) against the 5 selected antibiotic resistant bacteria.

Table 2: Mean inhibition zone diameter (mm) in different standard antibiotics by the disc diffusion method for susceptibility testing of selected bacterial strains

Bacterial strain	**Standard antibiotics (µg/disc)**						
	GM (10)	**E (15)**	**S (10)**	**P (10)**	**TE (30)**	**SXT (1.25 /23.75)**	**TMP (5)**
Staphylococcus aureus	19	24	13	26	21	24	20
Susceptibility of *S. aureus*	S ≥ 15	S ≥ 23	12 ≤ I ≤ 14	R ≤ 28	S ≥ 19	S ≥ 16	S ≥ 16
Bacillus cereus	20	28	18	NA	20	NA	NA
Susceptibility of *B. cereus*	S ≥ 15	S ≥ 23	S ≥ 15	R	S ≥ 19	R ≤ 10	R ≤ 10
Escherichia coli	22	9	18	12	25	8	7
Susceptibility of *E. coli*	S ≥ 15	R ≤ 13	S ≥ 15	R ≤ 14	S ≥ 19	R ≤ 10	R ≤ 10
Salmonella enteritidis	19	10	12	NA	17	26	26
Susceptibility of *S. enteritidis*	S ≥ 15	R ≤ 13	12 ≤ I ≤ 14	R ≤ 14	15 ≤ I ≤ 18	S ≥ 16	S ≥ 16
Klebsiella pnomoniae	23	19	19	7	17	29	28
Susceptibility of *K. pnomoniae*	S ≥ 15	14 ≤ I ≤ 22	S ≥ 15	R ≤ 14	15 ≤ I ≤ 18	S ≥ 16	S ≥ 16

GM: gentamicin, E: erythromycin, S: streptomycin, P: penicillin, TE: tetracycline,
SXT: sulfamethoxazole/trimethoprim, TMP: trimethoprim
NA: no antibacterial activity
R: resistance, I: intermediate susceptibility, S: susceptibility

The results of determination of MIC and MBC (Tables 3 and 4) indicated that *M. officinalis* essential oil had antibacterial activity against the 5 tested bacterial strains. Investigation on growth of selected bacterial strains in different concentrations of *M. officinalis* essential oil at the stage MBC (Table 3) showed that 0.31 µL/mL was minimum concentration of the essential oil that *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pnomoniae* did not grow. Also, 10 and 20 µL/mL was minimum concentrations of the essential oil that *Salmonella enteritidis* and *Bacillus cereus* did not grow, respectively. The minimum concentrations were determined as MBCs (Table 4). MICs were determined ≤ the same concentrations (Table 4). The negative control did not show any bacterial growth. Therefore, the tested *M. officinalis* essential oil was sterile. The positive controls showed bacterial growth. Therefore, DMSO had no antibacterial activity and bacterial strains were able to grow, respectively.

Table 3: Investigation on growth of selected bacterial strains in different concentrations of *M. officinalis* essential oil at the stage MBC

Bacterial strain	Different concentrations of the essential oil (µL/mL)											
	0.31	0.62	1.25	2.5	5	10	20	40	80	CON-	CON+11	CON+12
S. aureus	-	-	-	-	-	-	-	-	-	-	+	+
B. cereus	+	+	+	+	+	+	-	-	-	-	+	+
E. coli	-	-	-	-	-	-	-	-	-	-	+	+
S. enteritidis	+	+	+	+	+	-	-	-	-	-	+	+
K. pnomoniae	-	-	-	-	-	-	-	-	-	-	+	+

CON-: negative control, CON+11: positive control, DMSO. CON+12: positive control, no DMSO and essential oil
+: bacterial growth, -: no bacterial growth

Table 4: Determination of MIC and MBC of *M. officinalis* essential oil against selected bacterial strains

Bacterial strain	MIC (µL/mL)	MBC (µL/mL)
Staphylococcus aureus	≤ 0.31	0.31
Bacillus cereus	≤ 20	20
Escherichia coli	≤ 0.31	0.31
Salmonella enteritidis	≤ 10	10
Klebsiella pnomoniae	≤ 0.31	0.31

NA: no antibacterial activity

Some authors reported chemical composition of *M. officinalis* essential oil and their variation during different stages of the plant growth also, antioxidant, antimicrobial, antitumoral activities of the essential oil and using fields of them (De Sousa *et al.*, 2004; Mimica-Dukic *et al.*, 2004; Di Pasqua *et al.*, 2005; Bahtiyarca and Coşge, 2006; Hăncianu *et al.*, 2008; De Martino *et al.*, 2009; Gutierrez *et al.*, 2009; Stanojevic *et al.*, 2010; Saeb and Gholamrezaee, 2012; Abdellatif *et al.*, 2014). These articles show that the main components of *M. officinalis* essential oil are 39% citronellal, 33% citral (citronellol, linalool) and 2% geranial. In addition, this oil contains such as threeterpinene, phenol carbon-acid (rosmarinic acid), and flavonglychoside acids in low ratio. There are also caffeic acid (a kind of tannin), several flavonoids (luteolin-7-O-glucoside, isoquercitrin, apigenin-7-Oglucoside, and rhamnocitrin), rosmarinic acid, ferulic acid, methyl carnosoate, hydroxycinnamic acid, and 2-(3', 4'-dihydroxyphenyl)-1, 3-benzodioxole-5-aldehyde and some other aldehydes: beta-caryophyllene, neral, and geranyl acetate.
Also, these articles indicate that Lemon balm is a potential medicinal and aromatic plant grown commonly most of our wild areas. Its essential oil is currently used in medicine and pharmacology (antitumor, antibacterial, antimicrobial, antihistaminic, antispasmodic and antioxidant, by means of its antiviral effect curing of the herpes antiulcerogenic, moderate Alzheimer's disease, modulation of mood and cognitive performance, stimulating the immune system (against anti HIV-1) and the heart, insect bites, painful menstruation, colds, headaches, mumps, insomnia, mild sedative and anti-depressant), in

food industry (using it's essential oil for food spoilage yeasts to extending the storage periods, in soft drinks industry because its fresh lemon tastes and herbal tea industry) and in cosmetic industry (containing hydrosol for curing dermatogical problems).

Our results showed that *M. officinalis* essential oil had antibacterial activity against the 5 selected food-borne pathogenic bacteria.

Bacterial resistance to antibiotics is still a growing problem. Recently, antibacterial plant products have used to control growth of resistant bacteria to antibiotics (Coisin *et al.*, 2012).

Also, our results indicated that *M. officinalis* essential oil can be used instead of some standard antibiotics against the 5 selected antibiotic resistant bacterial.

Conclusion

This study has shown that: 1. Susceptibility of the 5 tested bacterial strains to the most concentration of *M. officinalis* essential oil (20 µL/disc) by the disc diffusion method can be put in the following order: *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Klebsiella pnomoniae* > *Salmonella enteritidis*. 2. Susceptibility of the 5 tested bacterial strains to minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *M. officinalis* essential oil can be put in the following order: *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pnomoniae* > *Salmonella enteritidis* > *Bacillus cereus*. 3. *M. officinalis* essential oil can be used instead of antibiotics of erythromycin (15 µg/disc), penicillin (10 µg/disc), sulfamethoxazole/trimethoprim (1.25 µg/23.75 µg/disc) and trimethoprim (5 µg/disc) against the 5 tested bacterial strains that were resistance to them.

REFERENCES

Abdellatif F, Boudjella H, Zitouni A and Hassani A (2014). Chemical composition and antimicrobial activity of the essential oil from leaves of algerian *Melissa officinalis* L. *Excli Journal* **13** 772-781.

Bahtiyarca B and Coşge B (2006). The essential oil of Lemon balm (*Melissa officinalis* L.), its components and using fields**.** *Journal of the Faculty of Agriculture* **21** 116-121.

Coisin M, Burzo I, Ştefan M, Rosenhech E and Zamfirache MM (2012). Chemical composition and antibacterial activity of essential oils of three *Salvia* species, widespread in Eastern Romania. *Analele Ştiinţifice ale Universităţii* **58**(1) 51-58.

De Sousa AC, Sales A, Blank AF, Alves PB, Alviano CS and Gattass CR (2004). *Melissa fficinalis* L. essential oil: antitumoral and antioxidant activities. *Journal Pharm Pharmacol* **56** 677-681.

Di Pasqua R, Feo V, Villani F and Mauriello G (2005). In vitro antimicrobial activity of essential oils from Mediterranean Apiaceae, Verbenaceae and Lamiaceae against foodborne pathogens and spoilage bacteria. *Annals of Microbiology* **55**(2) 139-143.

Gutierrez J, Barry-Ryan C and Bourke P (2009). Antimicrobial activity of plant essential oils using food model media: efficacy, synergistic potential and interaction with food components. *Food Microbiology* **26**(2) 142-150.

Hăncianu M, Aprotosoaie AC, Gille E, Poiată A, Tuchiluş C, Spac A and Stănescu U (2008). Chemical composition and in vitro antimicrobial activity of essential oil of *Melissa officinalis* L. from Romania. *Revista medico-chirurgicală a Societăţii de Medici şi Naturalişti din Iaşi* **112**(3) 843-847.

Martino L, Feo V and Nazzaro F (2009). Chemical composition and in vitro antimicrobial and mutagenic activities of seven Lamiaceae essential oils. *Molecules* **14** 4213-4230.

Mimica-Dukic N, Bozin B, Sokovic M and Simin N (2004). Antimicrobial and antioxidant activities of *Melissa officinalis* L. (Lamiaceae) essential oil. *Journal of Agricultural and Food Chemistry* **52**(9) 2485-2489.

Saeb K and Gholamrezaee S (2012). Variation of essential oil composition of *Melissa officinalis* L. leaves during different stages of plant growth. *Asian Pacific Journal of Tropical Biomedicine* 547-549.

Stanojevic´ D, Čomic´ L, Stefanovic´ O and Solujic´ SS (2010). In vitro synergistic antibacterial activity of *Melissa officinalis* L. and some preservatives. *Spanish Journal of Agricultural Research* **8**(1) 109-115.