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Chemical Compositions and Antimicrobial Activities of Essential Oils of *Varthemia persica*, *Foeniculum vulgare* and *Ferula lycia*

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ABSTRACT

Aromatic and medicinal plants are widespread throughout world. The experiment was started in season 2010-2011. This experiment was conducted to study the chemical compositions and antimicrobial activities of essential oils of *Varthemia persica*, *Foeniculum vulgare* and *Ferula lycia*. Antibacterial effects of the extracts were tested on 6 Gram-positive and 9 Gram-negative human pathogenic bacteria. The major compounds in the leaves of *Varthemia persica*, *Foeniculum vulgare* and *Ferula lycia* were α -thujene, α -pinene, β -pinene, limonene, menthol, germacrene D, 1,8-cineole, fenchone, linalool, thymol and trans-anethole. The results of the Minimal Inhibiting Concentration (MIC) and Minimum Bactericidal Concentration (MBC) varied from one bacterium to another. The highest and broadest activity was shown by *Foeniculum vulgare* oil. Thymol and menthol possessed the highest antibacterial activity among the tested components. The results of this study indicated that essential oils of these plants possess some compounds with antimicrobial properties which can be used as antimicrobial agents in new drugs for treatment of infectious diseases.

Key words: Essential oil, chemical compositions, antimicrobial activities, aromatic plants, medicinal plants

INTRODUCTION

The essential oils medicinal and aromatic plants have shown pharmaceutical and antibacterial properties (Reische *et al.*, 1998; Sacchetti *et al.*, 2005; Mohsenzadeh, 2007; Kumar and Ganjewala, 2007; Jamshidi *et al.*, 2009). Ismail *et al.* (2011), Ahmad *et al.* (2005), Madsen and Bertelsen (1995), Sawamura (2000), Ganjewala and Luthra (2007a, b), Reza and Abbas (2007), Swamy and Rao (2008), Soltan *et al.* (2009), Louis *et al.* (2011), Patra (2011) and Upadhyay and Patra (2011) reported that the essential oils of medicinal and aromatic plants have antimicrobial and antifungal properties. *Varthemia persica* is an aromatic plant in Iran and is belongs to the genus *Varthemia* (Mozaffarian, 1996; Gahraman and Attar, 1998; Ghasemi *et al.*, 2003). There is no report on the pharmacological activity of this plant, but antibacterial, antispasmodic and hypoglycemic effects have been reported for other spices (Afifi *et al.*, 1997; Aburjai *et al.*, 2001). *Ferula lycia* belongs to the Apiaceae family and is one of the most important genera in Iran. Kartal *et al.* (2007) and Kose *et al.* (2010) reported that essential oils *Ferula* species have antimicrobial and antioxidant activity. Akgul and Bayrak (1988) and Arslan *et al.* (1989) reported that essential oils Fennel have

antimicrobial and antioxidant properties. Similarity, Gulfranz *et al.* (2008) reported that essential oils *Foeniculum vulgare* have pharmaceutical, pharmaceutical, antibacterial properties. The objective of this research was to study chemical compositions and antimicrobial activities of essential oils of *Varthemia persica*, *Foeniculum vulgare* and *Ferula lycia*.

MATERIALS AND METHODS

The leaves of *Varthemia persica* and seed of *Ferula lycia* and *Foeniculum vulgare* have been collected during March-August 2010 in Iran. Then the plants were isolated from the other specimen and conserved for extraction. The essential oils were extracted by hydrodistillation using an apparatus of Clevenger. For this, mixing 250 g of plants was used in 1600 mL of distilled water. The extraction took 3 h. After filtration, the solvent is eliminated by reduced pressure distillation in rotary evaporator and pure oil was stored at 4°C in obscurity till the beginning of analysis. GC analysis was performed, using a Shimadzu GC-9A gas chromatograph equipped with a DB-5 fused silica column (30 m×0.25 mm i.d., film thickness 0.25 µm). The oven temperature was held at 50°C for 5 min and then programmed to 250°C at a rate of 3°C min⁻¹. Injector and detector (FID) temperatures were 290°C; helium was used as carrier gas with a linear velocity of 32 cm sec⁻¹. The percentages were calculated by electronic integration of FID peak areas without the use of response factors correction. Linear retention indices for all components were determined by co injection of the samples with a solution containing homologous series of C₈-C₂₂ n-alkanes. GC-MS analyses were carried out on a Varian 3400 GC-MS system equipped with a DB-5 fused silica column (30 m×0.25 mm i.d.); oven temperature was 40 to 240°C at a rate of 4°C. Transfer line temperature was 260°C. Carrier gas was helium with a linear velocity of 31.5 cm sec⁻¹, split ratio 1/60. In addition, ionization energy was 70 eV, scan time 1 sec and mass range 40-300 amu. Identification of components in the oil was based on retention indices relatives to n-alkanes and computer matching with the WILLEY 275. L library, as well as by comparison of the fragmentation patterns of mass spectra with those reported in the literature (Adams, 2001). The chromatographic conditions were identical to those used for GC analysis.

Tests for antibacterial activity: The used microorganisms in the present study were six Gram-positive (*Staphylococcus aureus*, *Bacillus cereus*, *Bacillus megaterium*, *Bacillus subtilis*, *Sarcina lutea* and *Streptococcus-β*-haemolyticus) and nine Gram-negative (*Salmonella typhi*, *Shigella dysenteriae*, *Shigella shiga*, *Shigella sonnei*, *Shigella boydii*, *Escherichia coli*, *Klebsiella* sp., *Pseudomonas aeruginosa* and *Proteus* sp.) human pathogenic bacteria. The antibacterial assays were carried out by the disc-diffusion (Verpoorte *et al.*, 1983) and microdilution method (Daouk *et al.*, 1995; Hanel and Raether, 1988; Espinel-Ingroff, 2001) in order to determine the antibacterial activity of oils and their components against the human pathogenic bacteria. The bacterial suspensions were adjusted with sterile saline to a concentration of 1.0×10⁵ CFU mL⁻¹. The inocula were prepared daily and stored at 4°C until time of use. Dilutions of the inocula were cultured on solid medium to verify the absence of contamination and to check the validity of the inoculum.

Disc-diffusion test: Compounds were investigated by the disc diffusion using 4 mm filter discs. Bacteria were cultured overnight at 28°C in LB medium and then adjusted with sterile saline to a concentration of 1.0×10⁵ CFU mL⁻¹. The suspension was added to the top of agar (6 mL) and dissolved in Petri dishes (2 mL/agar plate) with solid peptone agar. Filter discs with essential oils

and main components ($1.0 \mu\text{g mL}^{-1}$) were placed on agar plates (1 disc per agar plate). After 24 h of incubation at 28°C for bacteria the diameter of the growth inhibition zones was measured. Streptomycin was used as a positive control and $1 \mu\text{L}$ was applied to the discs from stock solution (1 mg mL^{-1}). All tests were done in duplicate. Three replications were used for each oil and for each component (Sokovic *et al.*, 2009).

Microdilution test: The minimum inhibitory and bactericidal concentrations (MICs and MBCs) were determined using microtitre plates. The bacterial suspension was adjusted with sterile saline to a concentration of $1.0 \times 10^5 \text{ CFU mL}^{-1}$. Investigated Compounds were dissolved in broth LB medium ($100 \mu\text{L}$) with bacterial inoculum ($1.0 \times 10^4 \text{ CFU per well}$) to achieve the wanted concentrations ($0.02\text{-}15.0 \mu\text{g mL}^{-1}$). The microplates were incubated for 24 h at 28°C . The lowest concentrations without visible growth (at the binocular microscope) were defined as concentrations that completely inhibited bacterial growth (MICs). The MBCs were determined by serial sub-cultivation of $2 \mu\text{L}$ into microtitre plates containing $100 \mu\text{L}$ of broth per well and further incubation for 72 h. The lowest concentration with no visible growth was defined as the MBC, indicating 99.5% killing of the original inoculum. The optical density of each well was measured at a wavelength of 655 nm by Microplate manager 4.0 (Bio-Rad Laboratories) and compared with a blank and the positive control. Streptomycin was used as a positive control using the same concentrations as in the disc diffusion test. Three replications were used for each oil and each component (Sokovic *et al.*, 2009).

RESULTS AND DISCUSSION

The chemical compositions of *Varthemia persica* essential oil has been listed in Table 1, in which the percentages of components are given it. In total, sixty- seven volatile constituents, representing 78.08% of the total composition, were identified in the leaves oils (Table 1). The most abundant

Table 1: Chemical composition of essential oils investigated

Components	<i>Varthemia persica</i> (%)	<i>Ferula lycia</i> (%)	<i>Foeniculum vulgare</i> (%)
α -thujene	10.4	0	0
α -pinene	12.2	60.11	15.02
Sabinene	0.21	1	0
β -pinene	0.10	20.03	1
Myrcene	0.10	0.99	2
α -Phellandrene	0.07	2	0
δ -3-Carene	0.01	0	0.57
α -Terpinene	0.52	0	0.45
ρ -Cymene	0.12	0	1.56
Limonene	0.90	6.32	0
γ -Terpinene	0.87	0	2.01
Terpinolene	0.22	0	0
Isopentyl isovalerate	0.01	0	0
Menthol	20.5	0	0
Terpin-4-ol	0.21	0	0
Decanal	0.20	0	0
Octanol acetate	0.11	0	0
Sabinene hydrate acetate	0.10	0	0
Decanol	0.33	0	0
Bornyl	0.12	0	0

Table 1: Continue

Components	<i>Varthemia persica</i> (%)	<i>Ferula lycia</i> (%)	<i>Foeniculum vulgare</i> (%)
Acetophenone	0.02	0	0
Tridecane	0.08	0	0
Undec-9-en-1-al	0.03	0	0
Sesamol	0.02	0	0
Limonene aldehyde	0.18	0	0
α -Cubebene	0.17	0.07	0
α -Ylangene	0.63	0	0
α -Copaene	0.80	0.32	0.34
β -Bourbonene	0.90	0.05	0
Longifolene	0.23	0	0
β -Elemene	0.21	0	0
Trimenal	0.41	0	0
Tetradecane	0.29	0	0
α -Cedrene	0.25	0	0
Caryophyllene trans(E)	0.91	0	0
β -Gurjunene	0.90	0	0
γ -Elemene	0.33	0	0
Aromadendrene	0.50	0.05	0
α -Humulene	0.92	0	0
Allo-Aromadendrene	0.93	0	0
Germacrene D	6.05	0.09	0
β -Selinene	0.55	0	0
Bicyclogermacrene	0.90	0.36	0
α -Muurolene	0.89	0.05	0
γ -Cadinene	0.90	0	0
δ -Cadinene	0.87	0	0
Artedouglosia oxide A	0.80	0	0
α -Calacorene	0.90	0	0
Elemol	0.66	0	0
β -Calacorene	0.33	0	0
Spathulenol	0.74	0	0
Arteamuin alcohol	0.34	0	0
Cedrol	0.22	0	0
Himachalen oxide (β)	0.25	0	0
Cubenol (1,10-di-epi)	0.78	0	0
γ -Eudesmol	0.99	0	0
Cubenol	0.56	0	0
Selin-11-en-4- α -ol	0.63	0	0
β -Eudesmol	0.45	0	0
Khusinol	0.97	0	0
Cedrol-5-neo	0.96	0	0
Cedren-13-ol	0.78	0	0
β -Sinensal	0.45	0	0
α -Atlantone	0.25	0	0
Bisabolene	0.64	0	0
β -Bisabolen-12-ol	0.10	0	0
α -Atlantone	0.10	0	0
Camphene	0.00	0.63	0
β -phellandrene	0.00	0.08	0

Table 1: Continue

Components	<i>Varthemia persica</i> (%)	<i>Ferula lycia</i> (%)	<i>Foeniculum vulgare</i> (%)
Ocimene	0	0.26	0
Limonene oxide	0	0.53	0
Nonanal	0	0.41	0
Pulegone	0	0.05	0
Trans-pinocarveol	0	0.57	0
Camphenol	0	0.55	0
D-verbenone	0	0.17	0
Isoborneol	0	0.47	0
Myrtenol	0	0.61	0
Fenchyl acetate	0	0.01	0
Bornyl acetate	0	0.87	0
Myrtenyl acetate	0	0.17	0
β -caryophyllene	0	0.81	0.21
α -ylangene	0	0.05	0
Aristolene	0	0.09	0
Methyl butanol	0	0	0.14
1,8-Cineole	0	0	8.36
Fenchone	0	0	8.13
Linalool	0	0	3.21
Methyl chavicol	0	0	1.12
Thymol	0	0	17.21
Trans-anethole	0	0	25.21
Total	78.07	97.77	86.54

Total identified constituents, percentages are mean of three replications obtained from electronic, measurements using Flame Ionization Detection (FID), 0: Not detected

components found in the leaf oil were germacrene D (6.05%), followed by menthol (20.05%), α -thujene (10.4%) and α -pinene (12.2%). Other components were present in amounts less than 1% (Table 1). The essential oils yield of *Varthemia persica* collected from Iran was 0.9%. The phytochemistry revealed that this leaves had compositions similar to those of other *Varthemia persica* essential oils analyzed by Ghasemi *et al.* (2003). The chemical compositions of essential oils of *Ferula lycia* are presented in Table 1. In total, thirty volatile compounds, representing 97.77% of the total composition, were identified in the seed oils (Table 1). The most abundant components found in the seed oil were α -pinene (60.11%), other predominant components were Sabinene (1%), β -pinene (20.03%) and limonene (6.32%). Kose *et al.* (2010) investigated the essential oil related to Turkey and found β -pinene (19.01%), α -pinene (59.81%) and limonene (3.21%) as main constituents. The chemical compositions of *Foeniculum vulgare* essential oil has been listed in Table 1, in which the percentages of components are given it. Sixteen components representing 86.54% of the total oil were characterized. The most prominent component of oil was α -pinene (15.02%), β -pinene (1%), myrcene (2%), ρ -cymene (1.56%), γ -terpinene (2.01%), 1,8-cineole (8.36%), fenchone (8.13%), linalool (3.21), methyl chavicol (1.12%), thymol (17.21%) and trans-anethole (25.21%) were the other main constituents of the oil. According to GC-MS results of the essential oil of *Foeniculum vulgare* collected from Pakistan, 1,8-cineole (3.2%) ρ -cymene (2.0%), fenchone (6.9%) and trans-anethole (70.1%) were found as main constituents Gulfranz *et al.* (2008). The obtained results and screening of antibacterial activity of essential oil of *Varthemia persica*, *Ferula lycia* and *Foeniculum vulgare* are summarized in Table 2 and 3. The essential oils

Table 2: Antibacterial activity of essential oils (1.0 µg mL⁻¹) in disc-diffusion method

Bacteria	<i>Varthemia persica</i>	<i>Ferula lycia</i>	<i>Foeniculum vulgare</i>	Streptomycin
Gram positive				
<i>Staphylococcus aureus</i>	25	30	36	20
<i>Bacillus cereus</i>	25	30	30	15
<i>Bacillus megaterium</i>	21	30	35	20
<i>Bacillus subtilis</i>	22	27	30	20
<i>Sarcina lutea</i>	20	26	27	18
<i>Streptococcus-β</i> -haemolyticus	20	25	25	15
Gram negative				
<i>Salmonella typhi</i>	15	20	15	10
<i>Shigella dysenteriae</i>	15	20	15	15
<i>Shigella shiga</i>	15	20	20	12
<i>Shigella sonnei</i>	15	20	18	10
<i>Shigella boydii</i>	17	20	17	15
<i>Escherichia coli</i>	16	20	16	13
<i>Klebsiella sp.</i>	15	20	18	10
<i>Pseudomonas aeruginosa</i>	15	20	15	11
<i>Proteus sp.</i>	15	21	15	10

The data show the diameter of inhibition zone growth in mm, the diameter of paper disc was 6 mm

Table 3: Antibacterial activity of essential oils (MIC and MBC-µg mL⁻¹), microdilution method

Bacteria	MIC/MBC			
	<i>Varthemia persica</i>	<i>Ferula lycia</i>	<i>Foeniculum vulgare</i>	Streptomycin
Gram positive				
<i>Staphylococcus aureus</i>	1.5	0.5	0.5	3
	1.0	1.0	0.5	5
<i>Bacillus cereus</i>	2.0	1.0	1.0	1
	1.5	2.0	1.0	1
<i>Bacillus megaterium</i>	1.5	1.0	1.0	2
	0.5	1.0	1.5	2
<i>Bacillus subtilis</i>	1.5	0.5	1.0	1.5
	1.0	1.5	1.0	2
<i>Sarcina lutea</i>	1.0	1.0	1.5	2
	0.5	2.0	1.0	1
<i>Streptococcus-β</i> -haemolyticus	2.0	5.0	0.5	0.5
	2.0	3.0	0.5	2
Gram negative				
<i>Salmonella typhi</i>	2.0	1.5	2.0	3
	1.0	1.0	2.0	2
<i>Shigella dysenteriae</i>	1.0	4.0	1.0	4
	1.0	2.0	1.5	1
<i>Shigella shiga</i>	1.0	1.5	1.0	1.5
	1.0	1.0	7.0	2
<i>Shigella sonnei</i>	2.0	10.0	5.0	0.5
	5.0	7.0	3.0	5
<i>Shigella boydii</i>	1.5	8.0	2.0	2
	1.5	2.0	1.5	1
<i>Escherichia coli</i>	4.0	1.5	1.0	2
	3.0	1.0	0.5	3

Table 3: Continue

Bacteria	MIC/MBC			
	<i>Varthemia persica</i>	<i>Ferula lycia</i>	<i>Foeniculum vulgare</i>	Streptomycin
<i>Klebsiella sp.</i>	1.5	3.0	1.5	1.5
	2.0	2.0	0.5	4
<i>Pseudomonas aeruginosa</i>	1.5	1.5	1.0	4
	1.5	1.5	0.5	2

MBC: Minimum bactericidal concentration test, MIC: Minimum inhibitory concentration test

Table 4: Antibacterial activity of essential oils components (1.0 µg mL⁻¹) in disc-diffusion method

Bacteria	α-thujene	α-pinene	β-pinene	Limonene	Menthol	Trans-anethole	Fenchone	1,8-Cineole	Thymol	Streptomycin
Gram positive										
<i>Staphylococcus aureus</i>	20	20	20	20	25	20	20	20	38	20
<i>Bacillus cereus</i>	14	20	15	20	25	15	20	15	38	20
<i>Bacillus megaterium</i>	15	20	15	17	25	20	17	15	35	20
<i>Bacillus subtilis</i>	10	20	15	18	30	14	15	20	30	20
<i>Sarcina lutea</i>	10	20	15	16	25	20	15	18	32	15
<i>Streptococcus β-haemolyticus</i>	15	20	15	15	25	15	18	16	30	18
Gram negative										
<i>Salmonella typhi</i>	9	19	18	20	20	10	11	10	21	15
<i>Shigella dysenteriae</i>	9	18	17	20	20	10	14	10	21	15
<i>Shigella shiga</i>	9	15	15	20	20	10	15	10	20	15
<i>Shigella sonnei</i>	9	15	20	15	20	12	10	15	25	15
<i>Shigella boydii</i>	9	15	20	15	20	14	15	10	20	15
<i>Escherichia coli</i>	11	17	15	20	21	14	10	12	21	15
<i>Klebsiella sp.</i>	10	16	15	18	20	12	10	14	21	16
<i>Pseudomonas aeruginosa</i>	8	9	15	17	20	15	10	9	21	15
<i>Proteus sp.</i>	9	15	15	15	20	10	10	14	20	15

The data show the diameter of inhibition zone growth in mm, The diameter of paper disc was 6 mm

which showed the best antibacterial activity in disc-diffusion method were *Varthemia persica* (15.0-25.0 mm), *Ferula lycia* (20.0-30.0 mm) and *Foeniculum vulgare* (15.0-36.0 mm). Streptomycin at 1 µg disc⁻¹ showed inhibition zones in the range of 10.0-20.0 mm (Table 2). Good inhibition zones were also obtained for *Foeniculum vulgare* oils. It can be seen that essential oils of *Foeniculum vulgare*, *Varthemia persica* and *Ferula lycia* possess a higher antibacterial effect than streptomycin (Table 2). The essential oils of *Foeniculum vulgare*, *Varthemia persica* and *Ferula lycia* inhibited all the bacteria in very low concentrations. Oil of *Foeniculum vulgare* exhibited much higher antibacterial activity with the same MIC (0.5-0.5 µg mL⁻¹) and MBC (0.5-0.5 µg mL⁻¹). Streptomycin showed MIC at 3.0-2.0 µg mL⁻¹ and MBC at 5.0-4.0 µg mL⁻¹. From the obtained results it can be noticed that streptomycin, *Varthemia persica* and *Ferula lycia* possessed lower antibacterial activity than *Foeniculum vulgare* (Table 3). The results of antibacterial activity of essential oils components are presented in Table 4 and 5. α-thujene, 1,8-cineole and α-pinene inhibited bacterial growth of all bacteria and inhibition zones were 9.0-20.0 mm, trans-anethole and fenchone reacted slightly better (inhibition zones 10.0-20.0 mm), while streptomycin, limonene and β-pinene showed inhibition with zones of 15.0-20.0 mm. Strong antibacterial activity was noticed for menthol (20.0-30.0 mm) and especially for thymol (20.0-38.0 mm). It can be seen that thymol and menthol showed higher antibacterial activity than streptomycin and previous components (Table 4). α-thujene, 1,8-cineole, β-pinene and α-pinene

Table 5: Antibacterial activity of essential oils components (MIC and MBC- $\mu\text{g mL}^{-1}$), microdilution method

Bacteria	MIC/MBC									
	α -thujene	α -pinene	β -pinene	Limonene	Menthol	Trans-anethole	Fenchone	1,8-Cineole	Thymol	Streptomycin
Gram positive										
<i>Staphylococcus aureus</i>	2.5	2.5	2.5	4	1	4	4	2.5	0.5	2
	2	2	2	5	1	5	5	2	1	2.5
<i>Bacillus cereus</i>	3	5	3	2	1	1.5	6	0.5	1.5	3
	2	3	2	1.5	2	3	2	2	1	3
<i>Bacillus megaterium</i>	5	3	4.5	2.5	1	1	4	0.5	1.5	2
	1	1.5	1	2	1.5	1	1	1	0.5	2.5
<i>Bacillus subtilis</i>	4	4	6	1.5	1	1	8	1.5	0.5	1.5
	4	2.5	2	1.5	1	0.5	7	1.5	0.5	4
<i>Sarcina lutea</i>	2	6	4	1.5	1	0.5	9	1	0.5	6
	2	5	1	1	1	0.5	10	1	1	2
<i>Streptococcus-β-haemolyticus</i>	1	2	2	1.5	1	0.5	5	1	1	4
	2.5	3	2	1.5	1	1.5	2	0.5	2	4
Gram negative										
<i>Salmonella typhi</i>	6	6	4	3	1.5	2	6	1	2	6
	2	9	5	5	1.5	1	8	2	3	7
<i>Shigella dysenteriae</i>	1	10	3	5	3	1.5	7	4	1.5	5
	5	6	2	5	2	3	4	3	1.5	2
<i>Shigella shiga</i>	9	4	1	4	1.5	3	9	5	1.5	4
	7	2	1	3	1	3	9	2	1.5	3
<i>Shigella sonnei</i>	5	1	1	3	1	1	6	4	0.5	5
	5	3	1	6	1	1	5	3	0.5	6
<i>Shigella boydii</i>	1	5	9	4	1	1	2	1	1	1.5
	1	8	5	4	1	1.5	5	4	1	2
<i>Escherichia coli</i>	2	4	1	4	1	1.5	6	2	1	3
	8	5	2	5	1	1.5	9	3	1	4
<i>Klebsiella sp.</i>	6	3	1	8	1	1	4	2	1	2
	2	2	2	2	1	2	2	2	0.5	2
<i>Pseudomonas aeruginosa</i>	4	5	1.5	3	2	1.5	3	1	1	1
	1.5	1.5	1.5	4	1	4	4	1.5	0.5	2

MBC: Minimum bactericidal concentration test, MIC: Minimum inhibitory concentration test

showed similar activity with MIC of 2.5-1.5 $\mu\text{g mL}^{-1}$ and MBC of 2.0-2.0 $\mu\text{g mL}^{-1}$. Limonene, trans-anethole and fenchone possessed moderate antibacterial activity with MIC 4.0-4.0 $\mu\text{g mL}^{-1}$ and MBC of 2.0-2.0 $\mu\text{g mL}^{-1}$. Menthol and thymol showed very strong antibacterial activity with MIC at 1.0-1.0 and 0.5-0.5 $\mu\text{g mL}^{-1}$, respectively, while bactericidal effects was achieved at MBC 1.0-1.0 $\mu\text{g mL}^{-1}$ for menthol and MBC 1.0-0.5 $\mu\text{g mL}^{-1}$ for thymol. Menthol and thymol showed higher antibacterial activity than streptomycin (MIC 2.0-2.0 and MBC 2.5-2.0 $\mu\text{g mL}^{-1}$) (Table 5). These results are similar to those found by (Ghasemi *et al.*, 2003; Kose *et al.*, 2010; Gulfranz *et al.*, 2008). Higher concentration of trans-anethole (70.1%) was found in fennel oil which contains both antioxidants and antimicrobial activities (Muckensturm *et al.*, 1997). While, Patra *et al.* (2002) reported that anethole and its isomers are responsible for antimicrobial activities of fennel oil. There is no report on the pharmacological activity of this species, but antibacterial, antispasmodic and hypoglycemic effects have been reported for other spices (Afifi *et al.*, 1997; Aburjai *et al.*, 2001). In a study about the antimicrobial activity of *Ferula szovitsiana*, it was found that *Bacillus subtilis* was the most sensitive to strain and the essential oil showed a weak activity against to all microorganisms (Dehghan *et al.*, 2007). Eftekhar *et al.* (2004) determined that the essential oil of

Ferula gummosa seeds showed an antibacterial activity against to *S. aureus*, *B. subtilis*, *Escherichia coli* and *E. faecalis* and a weak activity against to *Pseudomonas aeruginosa*. Another report showed that the essential oil of *Ferula latisecta* had significant activity against all Gram (+) bacteria and *E. coli* (Habibi *et al.*, 2006). The antimicrobial activities, in general have been mainly explained through terpenes with aromatic rings and phenolic hydroxyl groups able to form hydrogen bonds with active sites of the target enzymes, although other active terpenes, as well as alcohols, aldehydes and esters can contribute to the overall antimicrobial effect of essential oils (Belletti *et al.*, 2004). Essential oils are rich in phenolic compounds, such as thymol, are widely reported to possess high levels of antimicrobial activity (Belletti *et al.*, 2004). On the other hand, it has previously been shown that Carvone, Carvacrol and thymol are capable of inhibiting bacteria and fungi (Sokovic *et al.*, 2009). The essential oils containing terpenes are also reported which possess antimicrobial activity (Dorman and Deans, 2000), which are consistent with our present studies. The synergistic effects of these active chemicals with other constituents of the essential oils should be taken into consideration for the antimicrobial activity.

CONCLUSION

Essential oil of *Foeniculum vulgare*, *Ferula lycia* and *Varthemia persica* showed significant antimicrobial activity. α -pinene, α -thujene, limonene, 1,8-cineole, fenchone, thymol, trans-anethole and menthol were common in all the oils as eight major compounds. The results suggest that *Foeniculum vulgare*, *Ferula lycia* and *Varthemia persica* essential oils possess some compounds with antimicrobial properties, which can be used as antimicrobial agents in new drugs for treatment of infectious diseases. Moreover, the findings of this study demand further researches on the evaluation of antimicrobial properties of several phytochemicals and in particular fenchone and trans-anethole.

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