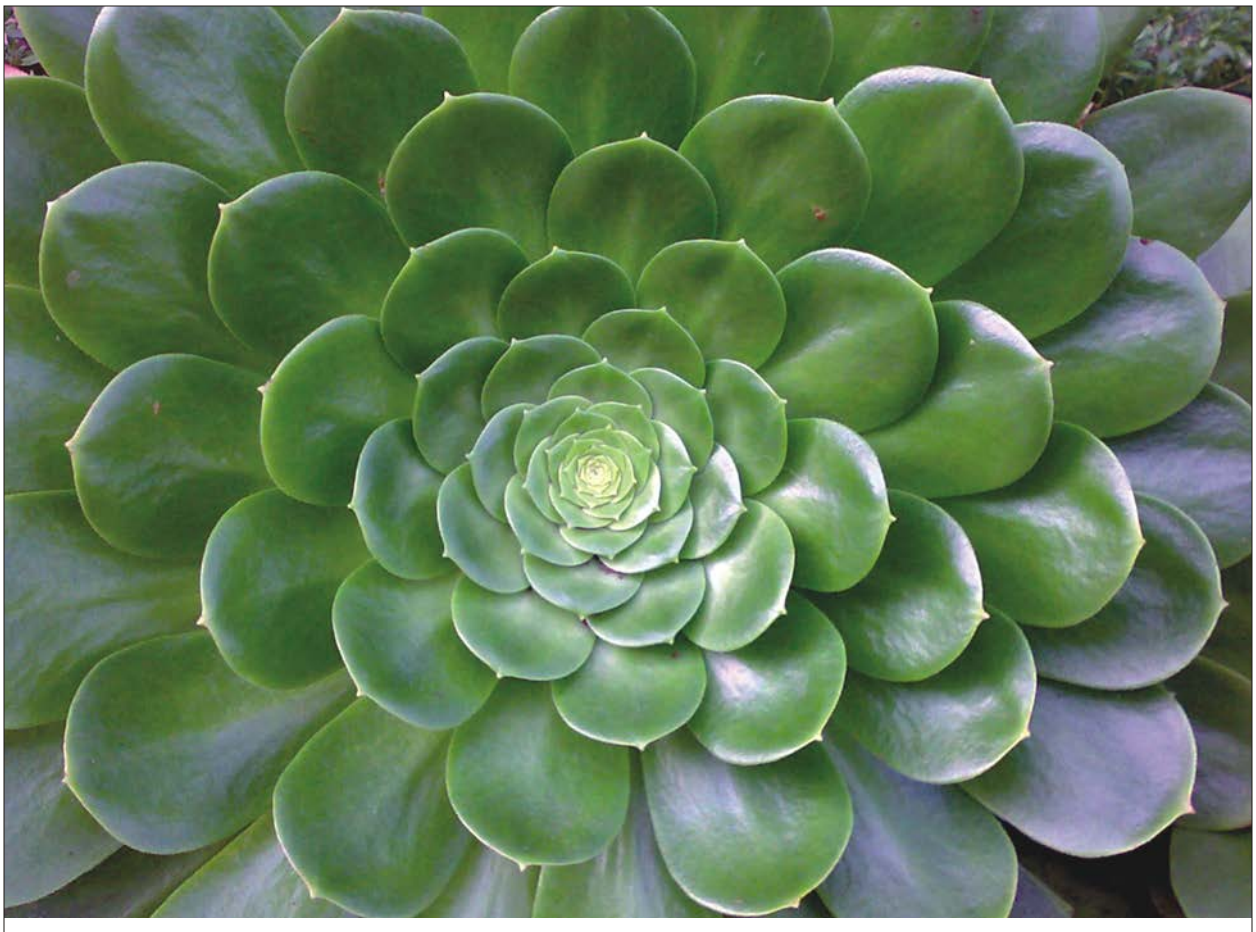

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Antibacterial and Antifungal Activity of some Medicinal Plants from Iran

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ABSTRACT

The objective of the research was to determine the chemical compositions and antibacterial and antifungal activity of essential oils from 4 medicinal plants consist of *Mentha piperita*, *Mentha spicata*, *Anethum graveolens* and *Foeniculum vulgare*. Antibacterial and antifungal activity of these oils and their components were assayed against a variety of human pathogenic bacteria. Main components in *Mentha piperita* oil were menthol, limonene, 1,8-cineole, sabinene, menthyl acetate and menthone, in *Mentha spicata* oil carvone, menthol, limonene and menthone, in *Anethum graveolens* leaves oil α-phellandrene, Dill ether and β-phellandrene and in *Foeniculum vulgare* oil terpin-4-ol, t-anethole, fenchone and estragole. Present results showed that oils extracted from leaves of *Anethum graveolens* and *Foeniculum vulgare* plants did not show antibacterial or antifungal activities. *Mentha piperita* showed strong antibacterial and antifungal activities, however, lower than *Mentha spicata*. Carvone possessed the highest antibacterial and antifungal activity among the tested components. Essential oils of *Mentha* species possess great antibacterial and antifungal potential and could be used as natural preservatives and fungicides.

Key words: *Mentha piperita*, *Mentha spicata*, *Anethum graveolens*, *Foeniculum vulgare*, antifungal activity

INTRODUCTION

Medicinal plants have pharmaceutical and antibacterial properties (Bari *et al*., 2010). Essential oils obtaining of different parts of Medicinal plants such as leave and seed (Ahmad *et al*., 2005; Ganjewala and Luthra, 2007a, b; Reza and Abbas, 2007; Swamy and Rao, 2008; Soltan *et al*., 2009; Fortes *et al*., 2011; Ismail *et al*., 2011; Louis *et al*., 2011; Patra, 2011; Upadhyay and Patra, 2011; Onocha *et al*., 2011). Antimicrobial properties of essential oils obtained from aerial parts and seeds of aromatic plants such as rosemary (*Rosmarinus officinalis*) and dill (*Anethum graveolens*) are well documented (Singh *et al*., 2002; Delaquis *et al*., 2002; Ruberto *et al*., 2000; Lo Cantore *et al*., 2004). Various studies have been published, investigating the antifungal and antibacterial activities of plant derived compounds against a range of pathogens (Tassou *et al*., 2000; Friedman *et al*., 2002; Momtaz and Abdollahi, 2010; Ara *et al*., 2009; Manikandan *et al*., 2011; Rahman *et al*., 2011; Ouattara *et al*., 2011). *Mentha piperita* and *Mentha spicata* used in the treatment of flatulent dyspepsia and intestinal colic due to its carminative and antispasmodic properties (Newall *et al*., 1996). Dill (*Anethum graveolens*) is from the family Umbelliferae and is an annual herb growing to a height of 1.5 m (Abed, 2007). Major compounds found in the essential

oil of dill includes furanocoumarin, 5-(4"-hydroxy-3"methyl-2"-butenyloxy)-6,7-furocoumarin, oxypeucedanin, oxypeucedanin hydrate and falcarindiol, all of reports indicated they have various degrees of antimycobactetial activity (Stavri and Gibbons, 2005). Fennel (*Foeniculum vulgare*) also belongs to the same botanical family, Umbelliferae. Extracts from fennel plant has been used as an antispasmodic, diuretic, analgesic and antipyretic and has antimicrobial properties; it can also be used for skin disorders, conjunctivitis and blepharitis of the eye (Ruberto *et al*., 2000; Ozbek *et al*., 2003). Moreover, screening of such plant extracts for antimicrobial and antifungal activities has always been of great interest to scientists looking for new sources for drugs for the treatment of various diseases (Oka *et al*., 2000). In this study, antimicrobial and antifungal activity of two *Mentha* species, Dill and Fennel oils was examined using different bacteria and fungi species. In addition, were determined chemical compositions of volatile constituents.

MATERIALS AND METHODS

The leaves of *Mentha piperita, Mentha spicata, Anethum graveolens* and *Foeniculum vulgare* have been collected during March-April 2010 in Iran (Ilam: Elevation 1339 m, Latitude East 33.638, Longitude North 46.431) 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 was 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 C_8-C_{22} 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 40-240°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 microorganisms used in the present study were 6 g positive (*Staphylococcus aureus, Bacillus cereus, Bacillus megaterium, Bacillus subtilis, Micrococcus luteus* and *Streptococcus*-β-*haemolyticus*) and 9 g negative (*Salmonella typhi, Shigella dysenteriae, Shigella shiga, Shigella sonnei, Shigella boydii, Escherichia coli, Klebsiella* sp., *Pseudomonas aeruginosa* and *Proteus* sp.) human pathogenic bacteria as well as eight pathogenic fungi (*Aspergillus fumigatus, Aspergillus niger, Aspergillus flavus, Vasin factum, Mucor* sp., *Candida albicans, Fusarium oxysporum* and *Colletotrichum falcatum*). 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 1.0×10^5 CFU mL^{-1}. The inocula were prepared daily and stored at +4°C until 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 1.0×10^5 CFU mL^{-1}. The suspension was added to the top of agar (6 mL) and dissolved in Petri dishes (2 mL $agar^{-1}$ plate) with solid peptone agar. Filter discs with essential oils and main components (1.0 μg mL^{-1}) were placed on agar plates (1 disc per agar plate). The fungi were grown in Potato Dextrose Agar (PDA) and/or Nutrient agar media. After 24 h of incubation at 28°C for bacteria or at 25°C for fungi the diameter of the growth inhibition zones was measured. Streptomycin was used as a positive control and 1 μL was applied to the discs from stock solution (1 mg mL^{-1}), whereas, antifungal drug is Nystatin at 50 μg $disc^{-1}$. All tests were done in duplicate; Three replications were done for each oil and for each component (Sokovic *et al*., 2007).

Microdilution test: The minimum inhibitory and bactericidal and fungicidal concentrations (MICs and MBCs) were determined using microtitre plates. The bacterial suspension was adjusted with sterile saline to a concentration of 1.0×10^5 CFU mL^{-1}. Compounds to be investigated were dissolved in broth LB medium (100 μL) with bacterial inoculum (1.0×10^4 CFU per well) to achieve the wanted concentrations (0.02-15.0 μg 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 μL into microtitre plates containing 100 μL 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, whereas antifungal drug is Nystatin at 50 μg $disc^{-1}$. Three replications were done for each oil and each component (Sokovic *et al*., 2007).

RESULTS AND DISCUSSION

The results of the chemical analyses of essential oils are presented in Table 1. In *Mentha spicata*, 25 volatile constituents, representing 96.45% of the total composition, were identified in the leaves oils (Table 1). The most abundant components found in the leaf oil were **β**-myrcene (4.01%), followed by limonene (10.1%), 1,8-cineole (2.16%), menthon (24.14%), menthol (6.12%) and carvone (40.11%). The yield of *Mentha spicata* oil is 2.8% (v/w). The essential oils yield of collected *Mentha piperita* from region of Iran (Ilam) was 3.02% and main components are sabinene (3.01%), limonene (8.16%), 1,8-cineole (6.31%), menthon (14.12%), menthofuran (4.12%), menthol (33.02%) and menthyl acetate (20.21%). Other components were presents in amounts less than 2% (Table 1). The chemical compositions revealed that these leaves had compositions similar to those of other *Mentha piperita* essential oils analyzed in Morocco by (Derwich *et al*., 2010) which the major component was menthol (29.1%), Menthol (5.58%), Menthyl acetate (3.34%) and Menthofuran (3.01%). Menthanol (36.24%) and menthone (32.42%). Also, menthanol (36.24%) and menthone (32.42%) were the major compounds of the *Mentha piperita* essential oil studied in Iran

Table 1: Chemical composition of essential oils investigated

Components	*Mentha spicata* (%)	*Mentha piperita* (%)	*Anethum graveolens* (%)	*Foeniculum vulgare* (%)
Tricyclene	0.52	0.00	0.00	0.00
α-thujene	1.00	0.00	1.00	0.00
Myristicin	0.00	0.00	3.31	0.00
α-pinene	1.01	0.00	1.90	0.63
Sabinene	1.60	3.01	1.11	0.00
β-pinene	0.68	0.00	0.54	1.73
β-myrcene	4.01	1.00	0.82	0.00
Carvacrol	0.00	0.00	0.00	0.30
3-octanol	0.00	0.60	0.00	0.00
t-anethole	0.00	0.00	0.00	17.19
α-terpinene	0.00	0.72	0.00	1.08
Estragole	0.00	0.00	0.00	3.18
α-Phellandrene	0.00	0.00	18.36	0.00
p-cymene	0.72	0.51	2.34	0.00
Limonene	10.10	8.16	1.12	0.21
β-Phellandrene	0.00	0.00	3.38	0.00
1,8-cineole	2.16	6.31	0.00	1.03
Cis-ocimene	0.00	1.03	0.00	0.00
Trans-ocimene	0.00	1.00	0.00	0.00
Fenchone	0.00	0.00	0.00	4.03
γ-terpinene	1.00	0.36	0.34	1.39
α-terpinolene	0.22	0.63	0.00	0.00
Linalool	0.00	1.00	0.00	0.00
α-p-Dimethyl-phencone	0.00	0.00	0.11	0.00
Phencone	0.00	0.00	0.20	0.00
Menthon	24.14	14.12	0.10	0.00
(Z)-p-menth-2-en-1-ol	0.00	0.00	0.10	0.00
Dill ether	0.00	0.00	5.02	0.00
Menthofuran	0.00	4.12	0.00	0.00
(Z)-Dihydrocarvone	0.00	0.00	0.12	0.00
Menthol	6.12	33.02	0.00	0.00
(E)-Dihydrocarvone	0.00	0.00	0.14	0.00
Terpin-4-ol	0.21	0.00	0.00	4.08
Cis-dihydrocarvone	0.22	0.00	0.00	0.00
Trans-dihydrocarvone	0.32	0.00	0.00	0.00
Trans-carveol	0.11	0.00	0.00	0.00
Carvone	40.11	0.00	0.00	0.00
(Z)-Anethole	0.00	0.00	0.46	0.00
Epi-bicyclosesquiphellandrene	0.00	0.00	0.34	0.00
Pulegone	0.30	0.00	0.00	0.00
Piperitone	0.44	0.62	0.00	0.00
Trans-anethole	0.40	0.00	0.00	0.00
Menthyl acetate	0.00	20.21	0.00	0.00
β-bourbonene	0.31	0.11	0.00	0.00
β-caryophyllene	0.31	0.10	0.00	0.00
(Z)-β-farnesene	0.00	0.32	0.00	0.00

Table 1: Continue

Components	*Mentha spicata* (%)	*Mentha piperita* (%)	*Anethum graveolens* (%)	*Foeniculum vulgare* (%)
Germacrene D	0.11	0.18	0.01	0.00
Bicyclogermacrene	0.00	0.92	0.00	0.00
Germacrene A	0.33	0.25	0.00	0.00
δ-cadinene	0.00	0.59	0.00	0.00
Apiol	0.00	0.00	0.80	0.00
2,3,4,6-tetramethyl-phenol	0.00	0.00	0.00	0.12
Timol	0.00	0.00	0.00	1.09
p-anisaldehyde	0.00	0.00	0.00	0.03
Carvacrol	0.00	0.00	0.00	1.03
Viridiflorol	0.00	0.10	0.00	0.00
Total	96.45	98.99	31.62	37.75

(Behnam *et al*., 2006). The chemical compositions of *Mentha piperita* L. essential oil studied in Iran, contained α-terpinene (19.7%), isomenthone (10.3%), trans-carveol (14.5%), pipertitinone oxide (19.3%) and β-caryophyllene (7.6%) as the major compounds (Yadegarinia *et al*., 2006). The chemical compositions revealed that the leaves had compositions similar to those of other *Mentha spicata* essential oils analyzed by (Sokovic *et al*., 2007), the major components was limonene (5.77%), Menthol (21.92%) and carvone (49.52%). Menthol and carvone were the main components of *Mentha piperita* (Derwich *et al*., 2010). Abbaszadeh *et al*. (2009), studying of essential oil compounds variations in leaves of *Mentha* species, indicated that was significant difference between essential oil yields in leaves of mint species. In the essential oil of *Anethum graveolens* leaves, 22 compounds were identified. Monoterpenic hydrocarbons were found predominant in the leaves oil representing 31.62% of the total content and main components are Myristicin (3.31%), α-Phellandrene (18.36%), Dill ether (5.02%) and β-Phellandrene (3.38%) which α-phellandrene (18.36%) constituted the major compound. Present result showed that in the leaves oil, the ketonic compound carvone was not present (Table 1). Principle components of fennel leaves oil were t-anethole (17.19%), estragole (3.18%), fenchone (4.3%) and terpin-4-ol (4.8%) (Table 1). The obtained results about antibacterial and antifungal activity of the leaves essential oils of *Mentha piperita* and *Mentha spicata*, *Anethum graveolens* and *Foeniculum vulgare* from Iran are shown on Table 2 and 3. All the tested oils in the disc-diffusion method showed bacteriostatic activity in concentration of 1 μg disc^{-1}, except of fennel (*Anethum graveolens*) and dill (*Foeniculum vulgare*) leaves oil. The tested essential oil of *Mentha piperita* and *Mentha spicata* by disc-diffusion method, showed very strong antibacterial and antifungal activity. A concentration of 1 μg disc^{-1} of oil inhibited *Staphylococcus aureus*, all of *Bacillus* species, *Salmonella typhi*, all of *Shigella* species, *Escherichia coli*, *Klebsiella* sp., *Pseudomonas aeruginosa*, *Proteus* sp., all of *Aspergillus* species, *Vasin factum*, *Mucor* sp., *Candida albicans*, *Fusarium oxysporum* and *Colletotrichum falcatum*. The essential oils which showed the best antibacterial and antifungal activity in disc-diffusion method were *Mentha piperita* (15.0-30.0 mm) and *Mentha spicata* (20.0-35.0 mm). Streptomycin at 1 μg disc^{-1} showed inhibition zones in the range of 9.0-22.0 mm (Table 2). Nystatin at 50 μg disc^{-1} showed inhibition zones in the range of 15-25.0 mm (Table 2). Good inhibition zones were also obtained for *Mentha spicata* and *Mentha piperita* oils. It can be seen that essential oils from *Mentha spicata* and *Mentha piperita* possess a higher antibacterial and antifungal effects than streptomycin and Nystatin. Table 2 shows that essential oil of *Mentha piperita* possesses greater antibacterial and fungistatic activity than *Mentha piperita* oil. *Anethum graveolens* and

Table 2: Antibacterial activity of essential oils (1.0 μg mL^{-1}) in disc-diffusion method, inhibition zones (mm)

Bacteria and fungi	*Mentha spicata*	*Mentha piperita*	*Anethum graveolens*	*Foeniculum vulgare*	Streptomycin	Nystatin
Gram positive						
Staphylococcus aureus	35	30	0	0	22	0
Bacillus cereus	35	25	0	0	20	0
Bacillus megaterium	35	25	0	0	20	0
Bacillus subtilis	32	25	0	0	20	0
Micrococcus luteus	30	25	0	0	15	0
Streptococcus-β-haemolyticus	25	25	0	0	15	0
Gram negative						
Salmonella typhi	21	15	0	0	20	0
Shigella dysenteriae	20	15	0	0	15	0
Shigella shiga	20	10	0	0	15	0
Shigella sonnei	20	10	0	0	15	0
Shigella boydii	22	12	0	0	15	0
Escherichia coli	20	14	0	0	15	0
Klebsiella sp.	22	15	0	0	12	0
Pseudomonas aeruginosa	24	15	0	0	10	0
Proteus sp.	25	15	0	0	9	0
Fungi						
Aspergillus fumigatus	20	20	0	0	0	25
Aspergillus niger	20	20	0	0	0	20
Aspergillus flavus	20	20	0	0	0	20
Vasin factum	20	20	0	0	0	15
Mucor sp.	20	15	0	0	0	22
Candida albicans	20	15	0	0	0	15

Foeniculum vulgare oil showed the lowest MIC (8.0-10.0 μg mL^{-1}) and MBC (7.0-12.0 μg mL^{-1}) in the microdilution method. The essential oils from *Mentha spicata* and *Mentha piperita* inhibited all the bacteria and fungi in very small concentrations. Oils from *Mentha* spicata (MIC (0.5-1.5 μg mL^{-1}) and MBC (1-1.0 μg mL^{-1}) and *Mentha piperita* (MIC (1.0-1.0 μg mL^{-1}) and MBC (1.5-1.0 μg mL^{-1}) exhibited much higher antibacterial and antifungal activity. Streptomycin showed MIC at 4.0-6.0 μg mL^{-1} and MBC at 5-3.0 μg mL^{-1} while, Nystatin showed MIC at 3.0-3.0 μg mL^{-1} and MBC at 4.0-2.0 μg mL^{-1}. From the obtained results it can be noticed that oils from *Anethum graveolens* and *Foeniculum vulgare* possessed lower antibacterial and antifungal activity than streptomycin and Nystatin while oils *Mentha spicata* and *Mentha piperita* showed almost the same antibacterial potential as the antibiotic (Table 3). The results of antibacterial activity of essential oil components are presented in Table 4 and 5. limonene inhibited bacterial growth of all bacteria and fungi and inhibition zones were 10.0-22.0 mm, 1,8-cineole reacted slightly better (inhibition zones 10.0-25.0 mm) while streptomycin showed inhibition with zones of 9.0-25.0 mm. Strong antibacterial and antifungal activity was noticed for menthol (20.0-35.0 mm), menthon (20.0-30.0 mm) and especially for carvone (20.0-38.0 mm). Streptomycin was inactive against all *Aspergillus* species, *Vasin factum, Mucor* sp., *Candida albicans, Fusarium oxysporum* and *Colletotrichum falcatum*. It can be seen that menthol, carvone and menthon showed higher antibacterial and antifungal activity than streptomycin and Nystatin. The monoterpenic hydrocarbons 1,8-cineole and linalool showed similar activity with MIC of 5.0-7.0 μg mL^{-1} and MBC of 6.0-10.0 μg mL^{-1}. Menthon and menthol showed very strong activity with MIC at

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

Bacteria	*Mentha spicata* (%) MIC/MBC	*Mentha piperita* (%) MIC/MBC	*Anethum graveolens* MIC/MBC	*Foeniculum vulgare* MIC/MBC	Streptomycin MIC/MBC
Gram positive					
Staphylococcus aureus	0.5	0.5	8	8.0	1.5
	1.0	1.0	7	7.0	1.0
Bacillus cereus	1.0	6.0	5	3.0	
	0.5	0.5	9	4.0	3.0
Bacillus megaterium	1.0	1.0	8	5.0	2.0
	1.5	1.5	9	4.0	2.0
Bacillus subtilis	1.5	1.5	8	4.0	2.0
	0.5	0.5	9	4.0	3.0
Micrococcus luteus	0.5	0.5	8	4.0	4.0
	0.5	0.5	9	4.0	5.0
Streptococcus-β-haemolyticus	2.0	2.0	8	1.5	4.0
	2.0	2.0	9	1.5	4.0
Gram negative					
Salmonella typhi	3.0	3.0	7	2.0	5.0
	4.0	4.0	8	1.5	5.0
Shigella dysenteriae	5.0	5.0	7	3.0	1.5
	5.0	5.0	7	5.0	1.5
Shigella shiga	5.0	5.0	7	1.5	1.5
	5.0	5.0	7	1.5	3.0
Shigella sonnei	4.0	4.0	7	2.0	2.0
	2.0	2.0	7	2.0	2.0
Shigella boydii	2.0	2.0	7	4.0	2.0
	2.0	2.0	7	4.0	2.0
Escherichia coli	1.5	1.5	7	4.0	1.5
	1.5	1.5	8	4.0	3.0
Klebsiella sp.	0.5	0.5	10	4.0	2.0
	0.5	0.5	8	4.0	2.0
Pseudomonas aeruginosa	0.5	0.5	8	4.0	1.5
	0.5	0.5	8	4.0	1.5
Proteus sp.	0.5	0.5	8	4.0	1.5
	0.5	0.5	8	4.0	1.5
Fungi					
Aspergillus fumigatus	1.0	1.0	10	8.0	3.0
Aspergillus niger	1.0	1.0	9	8.0	3.0
Aspergillus flavus	1.0	1.0	9	6.0	3.0
Vasin factum	1.0	1.0	8	7.0	3.0
Mucor sp.	1.0	1.0	8	7.0	3.0
Candida albicans	2.0	2.0	12	12.0	4.0
Fusarium oxysporum	0.5	0.5	7	7.0	2.0
Colletotrichum falcatum	1.5	1.5	10	10.0	3.0

1.5-3.5 μg mL^{-1} and 0.5-2.0 μg mL^{-1}, respectively while bactericidal effect were achieved at 1.5-1.0 μg mL^{-1} for menthon and 0.5-2.0 μg mL^{-1} for menthol. Carvone showed the strongest antibacterial and antifungal activity with MIC at 0.5-0.5 μg mL^{-1} and MBC at 0.25-1.0 μg mL^{-1}. Only menthon, menthol and carvacrol showed higher antibacterial activity than streptomycin

Table 4: Antibacterial activity of essential oils components (1.0 μg mL^{-1}) in disc-diffusion method, inhibition zones (mm)

Bacteria and fungi	Menthon	Limonene	1,8-cineole	Carvone	Menthol	Streptomycin	Streptomycin
Gram positive							
Staphylococcus aureus	30	22	25	38	35	25	0
Bacillus cereus	30	22	25	35	25	12	0
Bacillus megaterium	30	22	20	36	22	12	0
Bacillus subtilis	30	22	20	38	22	15	0
Micrococcus luteus	30	22	20	38	30	25	0
Streptococcus-β-haemolyticus	30	22	20	38	30	13	0
Gram negative							
Salmonella typhi	16	5	15	20	20	8	0
Shigella dysenteriae	14	5	15	22	21	9	0
Shigella shiga	15	5	10	22	23	10	0
Shigella sonnei	14	7	10	25	24	9	0
Shigella boydii	14	15	10	20	20	8	0
Escherichia coli	14	10	10	23	20	9	0
Klebsiella sp.	15	20	10	25	20	12	0
Pseudomonas aeruginosa	14	17	10	25	25	8	0
Proteus sp.	14	10	10	25	22	9	0
Fungi							
Aspergillus fumigatus	20	10	20	25	21	0	20
Aspergillus niger	20	15	20	26	20	0	15
Aspergillus flavus	22	15	20	26	22	0	10
Vasin factum	20	20	20	26	21	0	22
Mucor sp.	25	20	20	26	20	0	14
Candida albicans	25	20	20	26	20	0	16
Fusarium oxysporum	27	20	20	30	20	0	10
Colletotrichum falcatum	20	20	15	25	20	0	15

(MIC 3.0-3.0 μg mL^{-1} and MBC 5-4.0 μg mL^{-1}) and Nystatin (MIC 4.0-6.0 μg mL^{-1} and MBC 3-5.0 μg mL^{-1}) (Table 5). Carvone showed higher antifungal activities than previous components. It can be seen that essential oils from *Mentha spicata* possess a higher antibacterial and antifungal effect than *Mentha piperita*. The differences between antibacterial and antifungal activities of these two essential oils could be due to different chemical composition of essential oils. The greater antibacterial and antifungal potential of *Mentha piperita* essential oil could be explained by the presence of carvone which possesses very strong antifungal activity (Adam *et al*., 1998; Knobloch *et al*., 1988; Sokovic *et al*., 2009). The essential oil of *Mentha piperita* and *Mentha piperita* have menthol and 1,8-cineole as main components which also exhibited very good antifungal properties but lower than carvone. Carvone has better antifungal properties because of its high water solubility. One of the reasons for lower antifungal activity of *Mentha piperita* essential oil could be the large amount of menthyl acetate which causes a decrease of antifungal properties (Griffin *et al*., 2000; Sokovic *et al*., 2009). Both tested Mentha oils showed strong antibacterial activity against a variety of bacteria (Sokovic *et al*., 2007). According to present results, volatile oil extracted from leaves of both Fennel and Dill did not show any inhibition of the growth of the above mentioned test organisms. In a study by Lo Cantore *et al*. (2004) however, essential oil extracted from fennel showed a lesser antibacterial effect compared to coriander oil in inhibition of *Escherichia coli* and *Bacillus megaterium*. In agreement with present result

Table 5: Antibacterial activity of essential oils components (MIC and MBC μg mL^{-1}), microdilution method

Bacteria and fungi	Menthon MIC/MBC	Limonene MIC/MBC	1,8-cineole MIC/MBC	Carvone MIC/MBC	Menthol MIC/MBC	Streptomycin MIC/MBC
Gram positive						
Staphylococcus aureus	1.5	5.0	5.0	0.5	0.5	1.0
	1.5	3.5	3.5	0.25	0.5	2.5
Bacillus cereus	1.0	1.5	2.0	1.0	1.0	2.0
	2.0	2.0	4.0	0.5	1.5	1.0
Bacillus megaterium	2.0	2.0	6.0	0.5	1.5	1.0
	2.0	2.0	6.0	0.5	1.5	1.0
Bacillus subtilis	1.0	2.0	5.0	0.5	1.0	1.0
	0.5	2.0	4.0	0.5	1.0	1.0
Micrococcus luteus	1.0	2.0	5.0	0.5	1.0	1.0
	1.0	2.0	6.0	1.0	0.5	1.0
Streptococcus-β-haemolyticus	1.0	2.0	3.0	1.0	1.0	1.0
	1.0	2.0	5.0	0.5	0.5	1.0
Gram negative						
Salmonella typhi	1.5	5.0	9.0	1.0	1.5	2.5
	1.5	4.0	3.0	1.0	2.0	2.0
Shigella dysenteriae	3.0	3.0	5.0	1.0	2.0	3.0
	3.0	5.0	4.0	1.0	2.0	4.0
Shigella shiga	3.0	5.0	7.0	1.0	1.0	4.0
	2.0	4.0	7.0	1.0	2.0	5.0
Shigella sonnei	1.5	7.0	7.0	1.0	1.5	2.0
	2.0	9.0	7.0	1.0	1.5	2.0
Shigella boydii	2.0	9.0	7.0	1.0	2.0	4.0
	4.0	9.0	5.0	1.5	2.0	4.0
Escherichia coli	3.0	8.0	7.0	1.5	2.0	3.0
	3.0	7.0	7.0	4.0	2.0	3.0
Klebsiella sp.	3.0	5.0	7.0	3.0	2.0	1.5
	1.5	5.0	6.0	3.0	2.0	1.0
Pseudomonas aeruginosa	1.0	4.0	4.0	2.0	1.0	3.0
	1.0	5.0	5.0	3.0	2.0	1.5
Proteus sp.	1.0	5.0	5.0	1.5	2.0	1.0
	1.0	5.0	5.0	2.0	2.0	1.0
Fungi						
Aspergillus fumigatus	1.5	6.0	6.0	1.5	1.0	3.0
Aspergillus niger	2.0	4.0	6.0	1.0	0.5	3.0
Aspergillus flavus	3.0	2.0	6.0	1.0	0.5	4.0
Vasin factum	3.0	5.0	6.0	1.0	0.5	5.0
Mucor sp.	1.5	5.0	6.0	1.0	0.5	5.0
Candida albicans	1.0	10.0	10.0	1.0	2.0	5.0
Fusarium oxysporum	1.0	6.0	5.0	2.0	1.0	5.0
Colletotrichum falcatum	3.5	7.0	7.0	0.5	2.0	2.5

Abed (2007) reported that essential oil and leaves extracts of fennel and dill did not show any inhibition antimycobacterial and anticandidal activity. The results obtained by both methods suggested that menthon, menthol and carvacrol possessed greater antibacterial and antifungal activity than other investigated compounds.

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