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Chemical Compositions and Antibacterial Activity of the Essential Oils of *Thymus vulgaris* and *Tanacetum parthenium*

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ABSTRACT

Aromatic and medicinal plants are widespread throughout world. Essential oils obtained from different aromatic and medicinal plants parts have been shown antibacterial, antifungal, antiviral and antioxidant properties The experiment was started in season 2010-2011. This work was conducted for determination of the phytochemistry and antibacterial activity of the essential oil from leaves of *Thymus vulgaris* and *Tanacetum parthenium* growing in Iran. Antibacterial effects of the extracts were tested on six Gram-positive and nine Gram-negative human pathogenic bacteria. The major compounds in the leaves of *Thymus vulgaris and Tanacetum parthenium* were α-pinene followed by camphene, β- pinene, 1,8-cineole, camphor, thymol and carvacrol. 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 *Thymus vulgaris* oil. Thymol and carvacrol possessed the highest antibacterial activity among the tested components.

Key words: Antibacterial, thymol, carvacrol, *Thymus vulgaris*, *Tanacetum parthenium*

INTRODUCTION

The essential oils medicinal and aromatic plants have shown pharmaceutical, pharmaceutical, antibacterial properties (Bari *et al*., 2010). Thyme (*Thymus vulgaris* L.) is a plant belonging to the Lamiaceae family (Matiljan, 2008). Atti-Santos *et al*. (2004) reported that *Thymus vulgaris* oil have to act as antioxidant, antimicrobial agent and antifungal properties (Inouye *et al*., 2001; Hernandez *et al*., 2004; Boskabady *et al*., 2006; Bolukbasi and Erhan, 2007; Abu-Darwish and Abu-Dieyeh, 2009). Ismail *et al*. (2011), Ahmad *et al*. (2005), Ganjewala and Luthra (2007a, b), Reza and Abbas (2007), Musyimi and Ogur (2008), Swamy and Rao (2008), Imelouane *et al*. (2009), Soltan *et al*. (2009), Abd El-Mageed *et al*. (2011), Fortes *et al*. (2011), Louis *et al*. (2011), Patra (2011) and Upadhyay and Patra (2011) reported that essential oils *Thymus* species have strong antibacterial, antifungal, antiviral, antiparasitic and antioxidant activities. *Tanacetum parthenium* is a medicinal plant that have essential oils and it is belongs to Asteraceae family (Bernath, 2000; Burt, 2004; Izadi *et al*., 2010). Composition of the oils extracted from the aerial parts of *Tanacetum parthenium* has also been reported by Izadi *et al*. (2010). This study evaluated the antimicrobial activities of *Thymus vulgaris* and *Tanacetum parthenium*, leaves essential oil against fifteen human pathogenic bacteria.

MATERIALS AND METHODS

The leaves of *Thymus vulgaris* and *Tanacetum parthenium* have been collected during March-August 2010 in western regions of Iran. 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 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°C 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 microorganisms used 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 of 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 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 1.0×10^5 CFU mL^{-1}. The suspension was added to the top of agar (6 mL) and dissolved in Petri dishes (2 mL agar $plate^{-1}$) 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). 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 μL 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 1.0×10^5 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. Three replications were used for each oil and each component (Sokovic *et al*., 2009).

RESULTS AND DISCUSSION

The chemical compositions of essential oils of *Thymus vulgaris* and *Tanacetum parthenium* are presented in Table 1. In total, thirty nine volatile compounds, representing 71.13% of the total composition, were identified in the leaves oils of *Thymus vulgaris* (Table 1). The main one being α-pinene (6.54%), camphene (10.12%), thymol (11.15%), 1.8-cineole (3.42%), β-pinene (3.91%) followed camphor (22.14%). Other predominant components were myrcene (1.24%), P-cymene (1%), Linalool (1.11%), borneol (1%) and terpinene-4-ol (1%). Other components were presents in amounts less than 1% (Table 1). The essential oils yield of *Thymus vulgaris* was 1.87%. Badi *et al*. (2004) and Jordan *et al*. (2006) found that wild-growing thyme in Jordan had higher concentrations of essential oil (5.40%) than recorded in Egypt (1.07%), Chili (0.39%), Iran (1.4%) and Belarusian (1.75%) (Karawya and Hifrawy, 1974; Hudaib and Aburjai, 2007; Khazaie *et al*., 2008). The chemical compositions revealed that this leaves had compositions similar to those of other thyme essential oils analyzed by Imelouane *et al*. (2009) which the major constituent in lab sample have been reported as α-pinene, camphene, followed camphor. Phytochemical studies have reported the occurrence of α-pinene, p-cymene and terpinene in thyme essential oil (Ozcan and Chalchat, 2004). The above mentioned results are agreed with those recorded by Jordan *et al*. (2006) who studied the oil composition of thyme of the same specie sample in Spanish, the major components quantified were 1,8-cineole, followed by terphenyl acetate, borneol, linalool, β-pinene, alphaterpineol and camphor. In total, eighteen volatile compounds, representing 66.07% of the total composition, were identified in the leaves oils of *Tanacetum parthenium* (Table 1). The essential oils yield of *Tanacetum parthenium* was 4.18%. It is relatively higher than other plants *Tanacetum parthenium* (3.3%) (Izadi *et al*., 2010) and *Tanacetum argyrophyllum* (3.13%) (Askari and Mirza, 1998). Some investigators have shown that the major constituents of the essential oils extracted from aerial parts of *Tanacetum parthenium* have been camphor (56.9%) followed by camphene (12.7%) and p-cymene (5.2%) (Akpulat *et al*., 2005). Camphor existed in the aerial parts of *T. aucheranum* (11.6%), *T. hiliophyllum* (28.1%), *T. argenteum* (14%) and *T. argyrophyllum* (22.3%) (Salamci *et al*., 2007; Tabanca *et al*., 2007; Omidbeigi, 2007; Askari, 2008). The results of antibacterial activity of tested essential oils are presented in Table 2, 3. The essential oils which showed the best antibacterial activity in disc-diffusion method were related to *Thymus vulgaris* (20.0-38.0 mm) and *Tanacetum parthenium* (20.0-35.0 mm). Streptomycin at 1 μg $disc^{-1}$ showed inhibition zones in the range of 10.0- 22.0 mm (Table 2). It can be seen that essential oils *Thymus vulgaris* and *Tanacetum parthenium* possess a higher

Table 1: Chemical composition of essential oils investigated

Component	Thyme (%)	*Tanacetum parthenium* (%)
Tricyclene	0.25	0
α-thujene	0.34	0
α-pinene	6.54	4.15
Camphene	10.12	10.12
β-pinene	3.91	0
Myrcene	1.24	0
α-terpinen	0.35	0
p-cymene	1	0.87
1,8-cineole	3.42	0
Trans beta ocimene	0.3	0
Gama terpinene	0.09	0
Cis-sabinene hydrate	0.31	0
Camphenilone	0.4	0
α-terpinolene	0.09	0
Linalool	1.11	0
α-thujone	0.36	0
Neo-alloocimene	0.75	0
Camphor	22.14	33.23
Borneol	1	0.31
Terpinene-4-ol	1	0.25
Para-cymen-8-ol	0.38	0
α-terpineol	0.65	0.25
Verbenone	0.09	0
Carveol	0.12	0
Carvarcrol metyl ethyl	0.43	0
Bornyl acetate	0.09	0.79
Thymol	8.65	8.16
α-copaene	0.71	0
β-bourbonene	0.86	0
Alpha-gurjunene	0.66	0
β-caryophyllene	0.72	0
Aromadendrene	0.087	0
α-elemene	0.62	0
Germacrene-d	0.78	0
Bicyclogermacrene	0.04	0
Spathulenol	0.59	0
Caryophyllene oxide	0.92	0
β-oplerenone	0.02	0
Benzaldehyde	0	0.02
Sabinene	0	0.12
α-terpinene	0	0.14
Limonene	0	0.12
Pinocarvone	0	0.32
Chrysanthenyl acetate	0	1.32
Carvacrol	0	5.56
β-caryophyllene	0	0.34
Total	71.137	66.07

Total identified constituents (%) are mean of three replications obtained from electronic measurements using flame ionization detection (FID), 0: Not detected

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

Bacteria	Thyme (%)	*Tanacetum parthenium* (%)	Streptomycin
Gram positive			
Staphylococcus aureus	38	35	20
Bacillus cereus	35	30	20
Bacillus megaterium	32	34	21
Bacillus subtilis	30	32	20
Sarcina lutea	30	30	22
Streptococcus-β-haemolyticus	30	30	18
Gram negative			
Salmonella typhi	25	26	10
Shigella dysenteriae	26	26	10
Shigella shiga	25	22	10
Shigella sonnei	25	21	12
Shigella boydii	25	21	10
Escherichia coli	25	20	11
Klebsiella sp.	22	20	15
Pseudomonas aeruginosa	24	25	14
Proteus sp.	20	25	10

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

antibacterial effect than streptomycin. *Thymus vulgaris* and *Tanacetum parthenium* oils exhibited much higher antibacterial activity with the same MIC (1-0.5 μg mL^{-1}) and MBC (0.5-0.5 μg mL^{-1}). Streptomycin showed MIC at 3.0-1.5 μg mL^{-1} and MBC at 2.0-4.0 μg mL^{-1}. The results of antibacterial activity of essential oil components are presented in Table 4, 5. β-Pinene and 1,8-cineole showed the lowest antibacterial activity among the tested components, with inhibition zones 6.0-18.0 mm; α-pinene and camphene possessed almost the same activity, with inhibition zones 10.0-25.0 mm. Camphor inhibited bacterial growth of all bacteria and inhibition zones were 15.0-30.0 mm, carvacrol reacted slightly better (inhibition zones 20.0-35.0 mm) while Streptomycin showed activity with inhibition zones 15.0-25.0 mm. Thymol showed inhibition with zones of 25.0-38.0 mm. β-pinene and 1,8-cineole and showed the lowest antibacterial activity in the microdilution method, MIC at 5.0-6.0 μg mL^{-1} and MBC at 7.0-10.0 μg mL^{-1}. α-pinene and camphor exhibited inhibitory activity at 2.0-1.5 μg mL^{-1} and was bactericidal at 1.5-3.0 μg mL^{-1} while, streptomycin exhibited inhibitory activity at 7.0-5.0 μg mL^{-1} and was bactericidal at 6.0-9.0 μg m L^{-1}. Among the seven essential oil components tested, thymol (MIC at 0.5-0.5 μg mL^{-1} and MBC at 1.0-1.5 μg mL^{-1}) and carvacrol (MIC at 1.0-1.5 μg mL^{-1} and MBC at 1.5-1.5 μg mL^{-1}) showed the highest activity. Many studies have reported that phenolic compounds in medicinal plants and herbs significantly contributed to their antioxidant and pharmaceutical properties (Cai *et al*., 2004; Shan *et al*., 2005; Wu *et al*., 2006). The antimicrobial activities of phenolic compounds may involve multiple modes of action. For example, essential oils degrade the cell wall, interact with the composition and disrupt cytoplasmic membrane (Ultee *et al*., 1999; Lambert *et al*., 2001), damage membrane protein, interfere with membrane integrated enzymes (Raccach, 1984), cause leakage of cellular components, coagulate cytoplasm, deplete the proton motive force, change fatty acid and phospholipid constituents, impair enzymatic mechanisms for energy production and metabolism, alter nutrient uptake and electron transport , influence the synthesis of DNA and RNA and destroy protein translocation and the function of the mitochondrion in eukaryotes (Raccach, 1984; Nychas, 1995). Borneol has been reported to have significant

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

	Thyme	*Tanacetum parthenium*	Streptomycin
Bacteria	MIC/MBC	MIC/MBC	MIC/MBC
Gram positive			
Staphylococcus aureus	1	1	3
	0.5	0.5	2
Bacillus megaterium	2	2	4
	1.5	2	2
Bacillus subtilis	1	1.5	1.5
	1	0.5	1
Sarcina lutea	1	0.5	0.5
	1	1.5	6
Streptococcus-β-haemolyticus	1	1	5
	1	1.5	1
Gram negative			
Salmonella typhi	2	2	5
	3	1	6
Shigella dysenteriae	5	1.5	7
	2	2.5	10
Shigella shiga	5	3	9
	4	4	5
Shigella sonnei	1.5	2	7
	2	1	8
Shigella boydii	4	5	3
	2	2	5
Escherichia coli	3	4	4
	5	5	6
Klebsiella sp.	4	6	2
	0.5	0.5	4
Pseudomonas aeruginosa	0.5	0.5	4
	0.5	0.5	1.5

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

antimicrobial activity (Tabanca *et al*., 2001; Vardar-Unlu *et al*., 2003). Pinene-type monoterpene hydrocarbons (α-Pinene and β-Pinene) are well-known chemicals having antimicrobial potentials (Dorman and Deans, 2000). The essential oils containing terpenes are also reported to possess antimicrobial activity (Dorman and Deans, 2000) which are similar with our present studies. The antifungal and antibacterial activity exhibited by *Thymus* genus essential oil has been demonstrated by several researchers (Karaman *et al*., 2001; Rasooli and Mirmostafa, 2003). This oil also showed very strong antibacterial activity against food spoilage bacteria (Sokovic *et al*., 2007). Feverfew essential oils affected on the growth of bacteria and *C. albicans* that potentially causes infection (Izadi *et al*., 2010). Significant difference was observed between Gram-positive and Gram -negative bacteria in terms of their susceptibility, so that Gram-positive bacteria were more sensitive to antimicrobial activity of feverfew essential oil. The higher sensitivity of Gram-positive bacteria may be explained according to their cell wall structure. The antimicrobial activity of the essential oil from *Tanacetum parthenium* may be associated with its major components such as Thymol, carvacrol and α-Pinene. In previous studies, the antimicrobial effect of these components has been confirmed (Sokovic *et al*., 2007; Izadi *et al*., 2010). Camphor is most commonly used

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

Bacteria	Camphor	α-Pinene	Camphene	β-Pinene	Carvacrol	1,8-Cineole	Thymol	Streptomycin
Gram positive								
Staphylococcus aureus	25	22	20	10	35	18	30	25
Bacillus cereus	28	21	25	15	35	18	35	20
Bacillus megaterium	30	20	20	18	32	15	35	24
Bacillus subtilis	25	25	20	14	35	15	38	23
Sarcina lutea	25	25	10	15	30	15	32	25
Streptococcus-β-haemolyticus	30	20	20	15	28	12	35	20
Gram negative								
Salmonella typhi	15	18	15	8	20	6	30	20
Shigella dysenteriae	20	20	14	7	25	10	30	15
Shigella shiga	22	15	10	5	22	9	25	18
Shigella sonnei	15	16	10	6	22	5	28	22
Shigella boydii	18	13	10	5	21	15	25	20
Escherichia coli	17	10	15	6	20	12	30	20
Klebsiella sp.	15	15	18	8	22	10	28	15
Pseudomonas aeruginosa	18	18	19	5	25	9	26	15
Proteus sp.	18	15	18	6	25	9	25	15

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

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

	Camphor	α-Pinene	Camphene	β-Pinene	Carvacrol	1,8-Cineole	Thymol	Streptomycin
Bacteria	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC
Gram positive								
Staphylococcus aureus	2	2	5	5	1	5	0.5	7
	1.5	1.5	7	7	1.5	7	1	6
Bacillus megaterium	3	3	8	5	2	5	2	9
	2	2.5	5	5	2	4	1	7
Bacillus subtilis	5	2	7	5	1.5	1	1	5
	1.5	2	4	5	0.5	1	1	5
Sarcina lutea	0.5	1.5	5	1	0.5	1	0.5	4
	1	1	2	1.5	1	1	0.5	8
Streptococcus-β-haemolyticus	1	0.5	6	6	1	1	1	9
	2	0.5	4	3	1	1	2	5
Gram negative								
Salmonella typhi	4	2	5	9	3	9	5	6
	5	2	6	5	5	10	3	8
Shigella dysenteriae	8	4	8	8	2	6	4	9
	6	5	4	10	4	8	2	10
Shigella shiga	2	6	2	12	2	5	1.5	5
	5	3	6	9	3	5	0.5	9
Shigella sonnei	10	0.5	10	5	3	9	1	9
	5	4	5	7	5	9	1	8
Shigella boydii	3	8	8	6	1.5	10	5	5
	6	10	7	8	5	7	9	6
Escherichia coli	9	6	4	5	9	8	2	8
	5	6	5	5	4	5	3	10
Klebsiella sp.	6	5	9	5	2	3	2	12
	3	3	4	10	1.5	10	1.5	9
Pseudomonas aeruginosa	3	4	7	5	3	4	1	7
	1.5	1.5	6	6	1.5	6	0.5	5

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

externally to relieve arthritic and rheumatic pains. It is often used in steam vaporizers to help control coughs by producing a local anesthetic action to the throat and to loosen congestion due to colds (Baser *et al*., 2001). α-Pinene is used by the fragrance industry as a starting material in the syntheses of terpineols, borneol and camphor (Bauer *et al*., 1997). Camphor and chrysanthenyl acetate were the main components of the essential oil of *Tanacetum parthenium* originated from England (Christensen *et al*., 1999) but in the region of Turkey (Adams, 2001), the main constituents of *Tanacetum parthenium* essential oil were camphor, camphene and p-cymene. In conclusion Present findings on the components of essential oil from the leaves of *Thymus vulgaris* and *Tanacetum parthenium* were in agreement with the previous report (Bendahou *et al*., 2008; Izadi *et al*., 2010). Essential oil of *Thymus* species and *Tanacetum* contain mainly aromatic monoterpenes, carvacrol, thymol and p-cymene and their activity are often attributed to these compounds (Daouk *et al*., 1995; Izadi *et al*., 2010).

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