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Essential oil composition and antibacterial activity of *Achillea millefolium* L. from different regions in North east of Iran

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Achillea millefolium **L. belongs to Asteraceae family and widely distributed in Mountainous and Steppe regions of Golestan province. The aerial parts of plant were collected from two different regions (700 to 2300 m) and the essential oils were obtained by hydro distillation. Essential oil compositions were analyzed by Gas chromatography-mass spectrometry (GC-MS). Different compounds were identified and their yield was 99.43 and 99.70%, respectively in Maraveh Tapeh and Charbagh regions. 1,8-cineole (18.6%), camphor (13.9%), borneol (9.4%), terpinolene (8.6%), γ-terpinene (6.8%) and thujone (4.3%) were the major components oil in 2300 m, whereas terpinolene (81%) followed by borneol (4.2%), β-pinene (3.5%) and chamazulene (2.9%) were identified in the flower oil in 700 m. The antibacterial activities were studied *in vitro* against 9 Gram positive and negative bacteria. The bacterial strains tested were found to be more sensitive to essential oil in which obtained in 2300 m and showed a very effective bactericidal activity with minimum inhibitory concentrations (MIC) ranging from 12.6 to 112 µg/ml. *S. epidermicis*, *S. aureus*, *B. cereus*, *E. faecalis* and *E. coli* were the most sensitive bacteria from oil of 2300 m, whereas *S. aureus* was the most sensitive bacteria, *B. cereus*, *E. coli* and *S. typhimorium* have moderate sensitivity and other bacteria were resistant to the oil of 700 m. *E. coli* and *E. faecalis* with 16.5 and 14.2 mm inhibition zone were sensitive Gram negative bacteria with MIC 22.8 and 63.8 µg/ml, respectively. Results demonstrate that the oil of *A. millefolium* L. can become potentials for controlling certain important Gram positive and negative bacteria which produces many infectious diseases, but their effective is varied in different region.**

Key words: *Achillea millefolium* L., essential oil, 1,8-cineole, terpinolene, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*.

INTRODUCTION

Aromatic herbs have been used for centuries as remedies for human infectious diseases. They are a rich valuable natural source of biologically active compounds and have been shown to possess antibacterial, anti-fungal, antiviral, insecticidal and antioxidant properties (Kordali et al., 2005; Nostro et al., 2000; Kawther, 2007).

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Over the years, the World Health Organization (WHO) advocated traditional medicine as safe remedies for both microbial and non-microbial diseases. According to the WHO in 2008, above 80% of the world's population rely on traditional medicine for their primary healthcare needs. So the increasing prevalence of multi drug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raise the specter of untreatable bacterial infections and add urgency to the search for new infection-fighting strategies (Pierangeli et al., 2009).

The genus *Achillea* L. (Asteraceae), with local name Maramboo, is represented by about 115 species found in the Northern hemisphere, mostly in Europe and Asia (Oberprieler et al., 2007). *Achillea* is represented in Turkey with 46 taxa, of which 25 are endemic, and in Iran with 19 species, of which 7 species are endemic (Mozaffarian, 2009). The name of the genus originates from the ancient use as a wound-healing remedy by the Trojan hero *Achilles* (Benedek et al., 2007). The aerial parts of *A. millefolium* L. a well-known species among other members of *Achillea*, are commonly used in European and Asian traditional medicine for the treatment of gastrointestinal disorders and hepatobiliary complaints, as well as for wound healing and skin inflammations (Jaradat, 2005; Albuquerque et al., 2007; Ugulu et al., 2009).

Many species of the *Achillea* are traditionally used in Iran as diuretic and menstrual regularity agents for wound healing, diarrhea, flatulence and abdominal pain (Ghorbani, 2005; Mazandarani et al., 2007; Fakir et al., 2009). Screening of antimicrobial activity of *Achillea* species essential oils showed the constituents of *Achillea* include monoterpens, sesquiterpene lactones, flavonoids, and phenolic acids (Kastner et al., 1993; Sosa et al., 2001; Lemmens-Gruber et al., 2006).

The antibacterial and antifungal activities of the essential oil of *A. nobilis* (Karamenderes et al., 2007) have been reported. *In vivo* anti-inflammatory activities and the acute toxicity of the ethanol extract prepared from these species have also been studied (Karabay-Yavasoglu et al., 2007). In the present study, we investigated the antibacterial activities of flower head essential oils prepared from *A. millefolium* L. in two different regions in North of Iran (Mountains and steppe rangelands from 700 to 2300 m above sea level).

The antimicrobial activity of the essential oil, aqueous, methanol and hexane–ether–methanol extracts of *A. millefolium* from Iran and Turkey have been previously reported and showed moderate activity against *Clostridium perfringens*, *B. cereus*, *Candida albicans*, *E. coli*, *Pseudomonas aeruginosa*, *S. aureus*, *Salmonella enteridis* and *Aspergillus niger* (Sokmen at al., 2004; Karaalp et al., 2009; Candan et al., 2003; Lotfipour et al., 2008). Although phytochemical data about essential oil of *A. millefolium* L. was limited, but some identified compounds for example, thujone, terpinolene, camphor, alpha-humulene, camphene, alpha-pinene, beta-caryophylene, cadinene, verbenone, 1,8-cineole and camphor were the main oil constituents which had good antibacterial activity against Gram positive and Gram negative bacteria (Benedek et al., 2007).

Golestan province in North of Iran is blessed with a rich source of aromatic plants, and throughout its long history, has accumulated a rich body of empirical knowledge of the use of medicinal plants for the treatment of many various infection diseases which have not been previously investigated for their chemical constituents and biological potentials. *A. millefolium* which grows in mountainous or steppe regions with cool dry climate of Golestan province is a potential source of essential oils. So, in this study we determinate the chemical constituents and evaluate the antibacterial activity of extracted essential oils from flowers of *A. millefolium* L. in blooming which was collected in two regions of Golestan province in North east of Iran (700 to 2300 m) against five Gram-negative: *E. coli, P. aeroginosa, Klebsiella pneumoniae, Shigella dysentria* and *salmonella typhi* and four Gram-positive*: S. aureus, Staphylococcus epidermidis, Enterococcus faecalis* and *B. cereus in vitro* conditions.

MATERIALS AND METHODS

Study area

The present study was carried out in Charbagh and Maraveh Tappeh (Kechik) in Gorgan (Golestan province). The Chaharbagh is located in Southeast of the province, in of latitude of 36° 37' 24" to 36° 34' 28" and longitude of 54° 35' 26" to 54° 24' 32" bordering the Alborz zone with Clay soils. Its average height is 600 to 2350 m from sea levels, semi-dry climate, rainfall of about 305 mm/year and a mean temperature -2/8°C (January to February) and 17/3°C (July to August). The Maraveh Tappeh is located in Northeast of the province in steppe area with latitude of 55° 57' 55" to 52° 57' 55" and longitude of 25° 46' 37" to 15° 42' 37", several steppe and semi-steppe ranges in hills and mountains bordering the Kopetdagh zone, with marn to calcareous marns in Loas soils reaching 620 to 1264 m from sea levels, semi-dry climate, rainfall of about 482 mm/year and a mean temperature 6/9°C (January to February) and 27/2°C (August to September) (Figure 1).

Plant material

The flowering aerial parts of *A. millefolium* L. were collected in August, 2008 from Chaharbagh Mountain at height of 2,300 m and Maraveh Tappeh hill at height of 700 m above sea level. The plant was identified at the Islamic Azad University of Gorgan branch. A voucher specimen was deposited at the Herbarium of the Institute of Medicinal plants, Faculty of Science, Islamic Azad University of Gorgan branch, Gorgan, Iran.

Isolation of essential oil

The plant samples in blooming stage were dried at room temperature for 7 days. The flowers were hydro distillated in a Clevenger-type apparatus for 2 h according to the method recommended in the British Pharmacopoeia. The oil was dried over

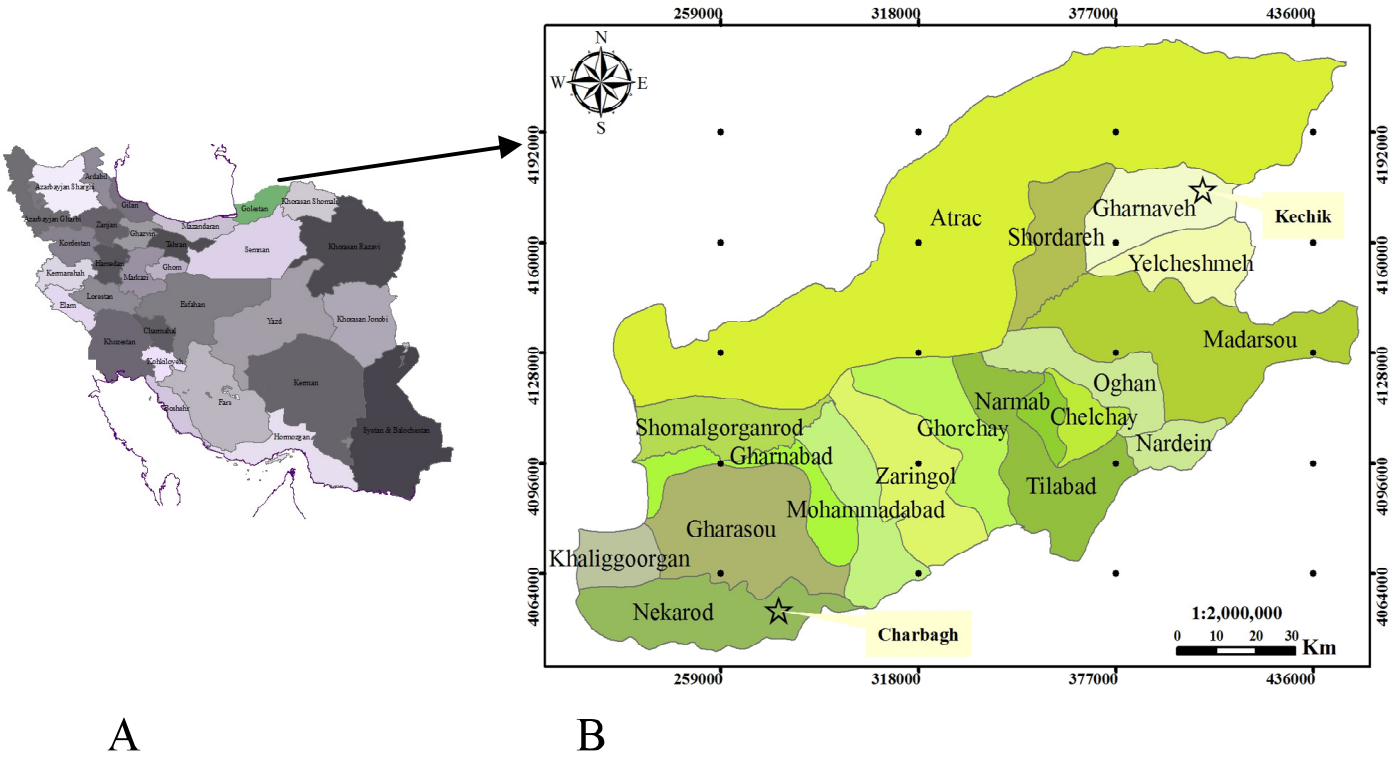

Figure 1. The locations of the studied area in Iran (A) and Golestan Province (B).

anhydrous sodium sulphate and deoxygenated under nitrogen gas and the oil obtained was kept refrigerated and protected from direct light until the analysis time.

Gas chromatography-mass spectrometry (GC-MS) analysis

The essential oil was analyzed by GC-MS. The GC-MS analysis was carried out on a Shimadzu GC-MS (QP5050). The capillary conditions were as follows; carrier gas, helium with a flow rate of 1.7 ml/min; injected 0.1 µL of the essential oil and ionization potential 70 ev. The initial temperature of column was 60°C (held for 1 min) then heated to 280°C with a rate of 3°C/min, then heated to 250°C and kept constant for 4 min. The same condition of temperature programming was used for n-alkenes mixture to calculate the retention indexes (RI). The identification of each component was studied by mass spectral data, literature and National Institute of Standards and Technology (NIST) computer library. The relative percentage of the oil constituent was calculated.

Bacterial strains

The bacterial strains were obtained from the Microbiology Laboratory, Golestan University of Medical Sciences. The essential oils of *A. millefolium* were individually tested against two strains of Gram positive and Gram negative bacteria: *Shigella dysenteria (*PTCC1188*), P. aeroginosa* (PTCC1430), *E. coli* (PTCC1399), *S. aureous* (PTCC1431), *B. cereus* (PTCC1015), *Salmonella typhimurium* (ATCC1596), *S. epidermidis* (PTCC1114), *Enterococcus faecalis* (PTCC1393) and *Kelebsiella pnumonie* (PTCC1291).

Evaluation of the antibacterial activity

At first screening, the essential oils were tested against the above mentioned bacteria. Minimal inhibitory conentrations (MICs) were determined by the agar serial dilution method at concentrations ranging from 0.93 to 60 µg/ml. Two fold serial dilutions were made from essential oil in molten Mueller Hinton agar (Pronadisa-Madrid) cooled to 45 to 50°C in a water bath. The essential oil was dispersed in mixture using dimethyl sulfoxide (DMSO). The amount of 0.01 ml of every bacterial suspension equivalent to McFarland tube No. 0.5 (108 CFU/ml) was inoculated on the agar of every well. The culture plates were then incubated at 37°C for 24 h. The MIC was defined as the lowest concentration at which no visible growth was observed (Hammer et al., 1999). The Mueller Hinton agar which contained DMSO without essential oil was used as negative control while gentamycine was used as positive control.

RESULTS AND DISCUSSION

Chemical composition of the essential oil

The essential oil analysis (Table 1) showed that variation in their quality and quantities due to essential oil of plant in Charbagh region led to approximately 25 compounds, with 99.7% yield (w/w) and 1,8-cineole (18.6%), camphor (13.9%), borneol (9.4%), terpinolene (8.6 %), γ-terpinene (6.8%) and thujone (4.3%), whereas terpinolene (81%) followed by borneol (4.2%), β-pinene (3.5%), chamazulene (2.9%), p-mentha-3,8-dinene (2.4%) and

Table 1. Essential oil composition of *A. millefolium* (L.) (700 to 2300 m).

Maraveh Tappeh (700 m)			Chaharbagh (2300 m)		
Component	**RI**	**Percent**	**Component**	**RI**	**Percent**
β-Pinene	985	3.5	α–Pinene	974	1.2
p-Mentha-3,8-dinene	1066	2.4	β-Pinene	986	2.1
Terpinolene	1080	81	Camphene	1012	1.3
Cis-thujone	1100	1.2	Sabinen	1040	3.2
Borneol	1176	4.2	α–Phelandrene	1066	0.9
Cis-sabinene hydrate cetate	1217	0.1	α–Terpinene	1086	3.4
Cis-carveol	1228	0.2	1,8-cineole	1100	18.6
Trans-carvone oxide	1267	0.4	γ-terpinene	1136	6.8
Bornyl acetate	1294	0.2	Thujone	1140	4.3
e-Caryophyllene	1442	0.6	Cis-thujone	1158	1.3
γ-Gurjunene	1488	0.5	Cis–mentha-2-en-1-ol	1168	0.6
Eugenolacetate	1511	0.7	Camphor	1170	13.9
e-Isoeugenol acetate	1613	0.5	Borneole	1186	9.4
(e,e)-Farnesol	1719	0.5	Terpinene-4-ol	1217	1.2
Chamazulene	1732	2.9	Terpinolene	1228	8.6
Unknown	1886	0.53	Trans-piperitol	1257	0.8
			7-Menthyl-3-menthylene-6-octan	1289	0.6
			Bornyl acetat	1294	1.3
			Caryophylene	1416	2.1
			Caryophylene-oxide	1426	1.6
			Nerodiol	1442	2.1
			Eujenol	1511	3.6
			Eujenol acetat	1539	1.2
			Farnesol	1717	2.3
			Chamazulene	1726	2.3
Total		99.43	Total		99.7

cis-thujone (1.2%) with 99.43% were the most components identified at 700 m (Maraveh tappeh).

According to similar reports, the essential oil of *A. millefolium* L. collected from Khorasan and Tehran Province (Iran) showed differences in their compound quantities, but their major components were simillar and included: α-pinene, 1,8-cineole, limonene, caryophyllene and camphor (Jaimand and Rezaee, 2004; Azizi et al., 2010). Somewhere else in North-west of Iran, imonene (14.94%), borneol (7.88%), α-cadinol (7.57%), caryophyllene oxide (4.34%) and terpinen-4-0l (3.89%) were the major components (Jaimand et al., 2000). In another research from two different regions, result showed that Trans-pinocarveol (29.2%), p-cymene (19.8%), limonene (18.2%), myrtenyl acetate (17%), n-heptanol (15.2%) and bornyl acetate (12%) were the major component in Damavand Mountain (Jaimand and Rezaee, 2004) in Elburse Mountain (Jaimand et al., 2006), chamazulene (54%), camphor (8%) and isoborneol (7.6%) were the major components. These results showed that the oils yield of *A. millefolium* and their components has been differently reported in previous studies confirmed by Afsharypour et al. (1996). In another report, essential oil composition of *A. millefolium* collected from the Plour in Mazandaran province is characterized by a high content of α-bisabolol (22.9%), spathulenol (12.4%) and cis-carveol (5%), whereas Rezaee et al. (2008) in Gilan province reported that the same plant included: cis-chrysanthenyl acetate (27.3%), hexyl butanoate (16%) and myrcene (7%).

In general, these findings confirmed that essential oil composition of plant can be different in quality and quantities in different geographical and environmental conditions and period of growth of plant.

Antibacterial activity

In the last few years, there has been target interest in biologically active compounds isolated from medicinal plants species for the elimination of pathogenic microrganisms because of the resistance that microorganisms have built against antibiotics (Essawi and Srour, 2000). Our results from antibacterial screening (Table 2) showed that *S. epidermisis* and *S. aureus* were the most sensitive bacteria to the plant oils from both region (33.6 to 31.4 mm)

Table 2. The average *in vitro* antibacterial activity and MIC values of essential oils from flowering aerial parts of *A. millefolium* L. (in well method) from two regions of Golestan province (Iran).

Microorganisms	Charbagh (2300 m)			Maraveh Tappeh (700 m)	
	Inhibition zone (mm)±SD	MIC (µg/ml)	Gentamycin	Inhibition zone (mm)±SD	MIC (µg/ml)
Staphylococcus aureus	31.4±0.8	15.4	6.7	19.8±0.3	27.5
Staphylococcus epidermidis	33.6±0.5	12.6	4.7	12.2±0.6	46.8
Bacillus cereus	22.1±0.1	29.2	6.5	12.7±0.1	45.3
Entrococcus faecalis	14.2±0.6	63.8	9.6	10.50.5	64.5
Escherichia coli	16.5±0.8	22.8	11	-	-
Pseudomonas aeruginosa	12.2±0.9	80.5	5.6	-	-
Klebsiella pneumonia	13.5±0.2	79.2	4.2	-	-
Salmonella typhymorium	12.3±0.3	112	18.8	-	-
Shigella dysentria	12.6±0.7	92.5	16	-	-

Disc diameter 6 mm average of two consecutive trials.

but the oil from higher region (2300 m) were best effective against tested bacteria, especially against *S. epidermisis, S. aureus, E. coli, B. cereus, E. faecalis* and *K. pneumonia* with MIC: 12.6, 15.4, 22.8, 29.2, 63.8 and 79.2 µg/ml, respectively, followed by the *B. cereus, E. coli, E. faecalis* and *K. pneumonia* which were found to be moderate sensitive bacteria with inhibition zone of 22.1, 16.5, 14.2 and 13.5 mm, respectively. Moderate antibacterial activities were observed against *P. aeruginosa, S. typhymorium* and *S. dysentria* with inhibition zones of 12.2, 12.3 and 12.6 mm and MIC of 80.5 to 112 µg/ml, respectively. Our results in Maraveh tappeh region showed that essential oil has lower effect on bacteria strains. As *S. aureus* with 19.8 mm inhibition zone is the most sensitive bacteria with the lowest MIC (27.5 µg/ml), *S. epidermicis and B. cereus* were moderately sensitive and others were resistant to the oil, exhibiting weak inhibition zones (10 to 12.2 mm) and high MIC values (above 45 µg/ml) in this region. The determination of the MIC by means of the liquid dilution method (Table 2) showed essential oil exhibited an antimicrobial effect against 9 tested bacteria, especially against Gram positive bacteria with MIC of 12.2 to 112 µg/ml in both regions.

Minimal inhibitory concentration (MIC), concentration range (12.6 to 112 µg/ml)

Briefly, results in Table 2 indicated that *S. aureus*, *S. epidermidis* and followed by *E. coli* were sensitive bacteria strains, especially against oil plant from Charbagh region. The compounds of plant essential oil in Charbagh region showed stronger antibacterial activity against tested bacteria contrast than Maraveh Tappeh, which were similar to other results (Karaalp et al., 2009). The *S. aureus* and *P. aeruginosa* have been applicable in cases of boils, sores and wounds and are considered as main pathogens causing hospitalized patients' infections (Braude, 1982). Also, the effectiveness of essential oil growth inhibition against *E. coli* justifies its use in the control of diarrhea and dysentery in humans (Ogbulie et al., 2007).In this research, the antibacterial activity of *A. millefolium* is due to some compounds in essential oil (Simic et al., 2005). The antibacterial activities of these essential oil could be associated to the presence of 1,8-cineole, camphor, borneol, terpinolene and γ-terpinene in Chaharbagh region and terpinolene in Maraveh Tappeh region as the most characteristic compounds of the essential oil of this plant. Among the essential oils, antimicrobial activities of camphor, 1,8-cineol, terpinen-4-ol and borneol in *Achillea* genus (Candan et al., 2003), 1,8-cineol in *Eucalyptus globules* (Tohidpour et al., 2010) have also been previously reported by other investigators especially against *S. aureus*, *P. aeruginosa* and *E. coli*.

Also in another work, terpinolene, terpinene 4-ol, γ-terpinene, 1,8-cineole, borneol, β-pinene, Chamazulene and thujone were the most active compounds of *A. millefolim*, *Thalictrum minus* and *Eucalyptus* spp. (Cimanga et al., 2002; Ogbulie et al., 2007; Lotfipour et al., 2008). These compounds have strong antibacterial activity as antiseptic, sedative, anti spasm, anti inflammation, expectorant and anti pathogen which have been used in traditional medicine of many countries to heal wounds and treat rheumatic pains, arthritis, urinary tract infection (UTI), cold, flu and leishmanious infection (Pieroni and Quave; 2005; Ugulu et al., 2009; Mendel and Hollis, 2010).

In many literatures, it is described that the antibacterial activity of *A. millefolium* can be dependent on variation of their terpenoids and flavonoids in essential oil or extract, their habitat and their period growth (Simoes et al., 1999; Lorenzi and Matos, 2002; Kotan et al., 2010). Through in this study we showed that when the same plants were grown in different regions (2300 to 700 m), their constituents were different and their medicinal activities

can be changed. Difference in these data compared to those previously reported in the literature that were collected in other geographic areas in Iran could be attributed to some ecological factors, the time of the growth and collection, model of extraction and etc. (Mazandarani et al., 2006; Haziri et al., 2010). According to the observed antibacterial activities, the basis of essential oil results showed that the *A. millefolium* growing in mountainous regions of Charbagh (2300 m) have potentially a good source of antimicrobial agent and support the traditional applications of *A. millefolium* as anti inflammation, sedative and anti pathogen for treatment of infectious diseases, healing wounds and boils. These results suggested the presence of either good antibacterial potency or of high concentration of an active principle in the essential oil. The results confirmed the application of essential oil plant in traditional medicine as an appropriate treatment for infectious disease. In previous reports, the antibacterial activity of this plant is reported from Iran without any data on MIC of this herb. Furthermore, our study showed activity of *A. millefolium* against both Gram positive and Gram negative bacteria in contrast with reports in which antibacterial activity was limited to Gram positive bacteria. Antibacterial activity somewhat is indicative of the presence of some antibacterial compounds, and many antibacterial agents have been originally derived from medicinal herbs (Ahmadian-Attari et al., 2009).

Conclusion

It can be concluded that essential oil of *A. millefolium* L. varied in its constituents, but 1,8-cineole, camphor, borneol, terpinolene,γ-terpinene and thujone were the most compounds of essential oil which can possess more antibacterial activity especially against Gram positive bacteria. Considering the increased development of resistance of bacteria to antibiotics, we believe that the present investigation together with previous studies provide support to the antibacterial properties of this plant oil. It can be used as antibacterial supplement towards the development of new therapeutic agents. Additional *in vivo* studies and clinical trials would be needed to justify and further evaluate the potential of this oil as an antimicrobial agent in topical or clinical applications, and further studies are necessary to evaluate the *in vivo* effects of active compounds of this plant. In addition, investigations confirm that higher plants used as anti infective phyto medicines may serve as a valuable source for novel antibiotics. Our results demonstrate that the oil of *A. millefolium* L. could become potentials for controlling certain important Gram positive and negative bacteria which produces many infectious diseases.

REFERENCES

Ahmadian-Attari MM, Monsef Esfahani HR, Amin GR, Fazeli MR (2009). The ethnopharmacological study on antibacterial activity of some selected plants used in Iranian traditional medicine. J. Med. Plant 8(31):50-57.

Albuquerque UP, Medeiros PM, Almeida ALS, Monteiro JM (2007). Medicinal plants of the Caatinga (semi-arid) vegetation of NE Brazil: A quantitative approach. J. Ethnopharmacol. 114:325-354.

Azizi M, Chizzola R, Ghani A, Oroojalian F (2010). Composition at different development stages of the essential oil of four *Achillea* species grown in Iran. Nat. Product Comm. 5(2):175-350.

Braude AI (1982). Microbiology. WB Saunders Company, Igaku-Shoin/Saunders, London. P 845.

Benedek B, Rothwangl-Wiltschnigg K, Rozema E, Gjoncaj N, Reznicek G (2007). Yarrow (*Achillea millefolium* L. s.l.): Pharmaceutical quality of commercial samples. Pharm. J. 63:23–26.

Candan F, Unlu M, Tepe B, Daferera D, Polissiou M, Sökmen A, Akpulat HA (2003). Antioxidant and antimicrobial activity of the essential oil and methanol extracts of *Achillea millefolium* subsp. *millefolium* Afan. (Asteraceae). J. Ethnopharmacol. 87: 215-220.

Cimanga K, Kambu L, Tona S, Apers S, De Bruyne T, Hermans N, Totté J, Pieters L, Vlietinck AJ (2002). Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo. J. Ethnopharmacol. 79:213–220.

Lorenzi H, Matos FJA (2002). Plantas Eedicinais no Brasil: Nativas e Exoticas. Instituto Plantarum, Nova Odessa, SP. pp. 129-130.

Essawi T, Srour M (2000). Screening of some Palestinian medicinal plants for antibacterial activity. J. Ethnopharmacol. 70:343–349.

Ghorbani A (2005). Studies on pharmaceutical ethnobotany in the region of Turkmen Sahra, north of Iran (Part 1): General results. J. Ethnopharmacol. 102:58-68.

Fakir H, Korkmaz M, Guller B (2009) . Medicinal plant diversity of Western Mediterrenean region in Turkey. J. Appl. Biol. Sci. 3(2):30-40.

Kastner U, Sosa S, Tubaro A, Breuer J, Rucker G, DellaLoggia R (1993) . Anti-edematous activity of sesquiterpene lactones from different taxa of the *Achillea millefolium* group. J. Planta Med. 59(l): A669.

Haziri AI, Aliaga N, Ismaili M, *et al* (2010). Secondary metabolites in essential oil of *Achillea millefolium* (L.) growing wild in East part of Kosova. Am. J. Biochem. Biotechnol. 6(1): 32-34.

Kawther FA (2007) . Antibacterial and Anticandidal Activity of Essential Oils of some Medicinal Plants in Saudi Arabia. Saudi J. Biol. Sci. 14(2):245-250.

Karamenderes C, Karabay-Yavasoglu NU, Zeybek U (2007). Composition and antimicrobial activity of *Achillea nobilis* L. subsp. *sipylea* and subsp. *neilreichii* essential oils from Turkey. J. Chem. Nat. Comp. 43:632–634.

Mozaffarian V (2009). A dictionary of Iranian Plant Names. Tehran, Farhang Moaser Pub. p 596.

Karabay-Yavasoglu U, Karamenderes C, Baykan S, Apaydin S (2007). Antinociceptive and anti-inflammatory activities and acute toxicity of *Achillea nobilis* subsp. *neilreichii* extract in mice and rats. J. Pharmaceut. Biol. 45:162–168.

Karaalp C, Yurtman AN, Yavasoglu NUK (2009). Evaluation of antimicrobial properties of *Achillea L.* flower head extracts. J. Pharmaceut. Biol. 47(1):86–91.

Kordali S, Kotan R, Mavi A (2005) . Determination of the chemical composition and anti oxidant activity of the essential oil of *Artemisia dracunculus* and *A. annuae*. J. Agric. Food Chem. 34(53, 24):9452-9458.

Kotan R, Cakir A, Dadasoglu F, Aydin T, Cakmakci R, Ozer H, Kordali S *(*2010). Antibacterial activities of essential oils and extracts of Turkish *Achillea, Satureja* and *Thymus* species against plant pathogenic bacteria. J. Sci. Food Agric. 15(90):145-60.

Jaimand K, Rezaei MB, Barazandeh MM (2000). Investigaion on essential oil composition of *Achillea millefolium* L. Subsp. Millefolium. J. Pajouhesh Sazandegi 13(48):68-69.

Jaimand K, Rezaei MB, Mozaffarian V (2006). Chemical constituents of the leaf and flower oils from *Achillea millefolium* ssp. Elbursensis Hub.-Mor. From Iran rich in Chamazulene. J. Essent. Oil Res.18:293-295.

Jaimand K, Rezaee MB (2004). Investigation on chemical constituents of essential oils from *Achillea millefolium* L. subsp. millefolium by distillation methods. Iranian J. Med. Aromatic Plants Res. 20(2):181-190.

Jaradat N (2005). Medical plants utilized in Palestinian folk medicine for treatment of diabetes mellitus and cardiac diseases. J. Al-Aqsa Univ. 9:1-28.

Lemmens-Gruber R, Marchart E, Rawnduzi P, Engel N, Benedek B, Kopp B (2006). Investigation of the spasmolytic activity of the flavonoids in yarrow (*Achillea millefolium*) on isolated guinea-pig ilea. J. Arzneimittelforschung 56(8):582-588.

Lotfipour F, Nazemiyeh H, Fathi-Azad F, Garaei N, Arami S (2008) . Evaluation of antibacterial activities of som medicinal plants from North-West Iran. Iranian J. Basic Med. Sci. 11(2):80-85.

Mazandarani M, Rezaei M B, Ghaemi FO, Ghaffari F (2006). Ethnobotany, chemical composition and antibacterial activity of the essential oil from *Achillea micranta* L. in Golestan province. J. Plant Sci. Res. 1(3): 48-53.

Mazandarani M, Yassaghi S, Rezaei MB, Ghaffari F (2007). Ethnobotany and anti bacterial activity from essential oil of two endemic *Hypericum* species in North of Iran. Asian J. Plant Sci. 6(2): 354-358.

Mendel J, Hollis A (2010). The Health impact Fund and Traditional Medicines. IGH Discussion Pap. 8:1-21.

Nostro N, Germano M, Angelo VD, Marino A, Cannatelli MA (2000). Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Lett. Appl. Microbiol. 30:379–384.

Pierangeli G, Vital G, Windell R (2009). Antimicrobial activity and cytotoxicity of *Chromolaena odorat*a (L. f). King and Robinson and Uncaria perrottetii (A. Rich) Merr. Extracts. J. Med. Plants Res. 3:511–518.

Hammer KA, Carson CF, Riley TV (1999). Antimicrobial activity of essential oils and other plant extracts. J. Appl. Microbiol. 86: 985-990.

Pieroni A, Quave CL (2005). Traditional pharmacopoeias and medicines among Albanians and Italians in southern Italy: A comparison. J. Ethnopharmacol. 101:258-270.

Ogbulie JN, Ogueke CC, Okoli IC, Anyanwu BN (2007). Antibacterial activities and toxicological potentials of crude ethanolic extracts of *Euphorbia hirta*. Afr. J. Biotechnol. 6(13):1544-1548.

Oberprieler Ch, Vogt R, Watson LE (2007). XVI. Tribe Anthemideae Cass. In: Kadereit JW, Jeffrey C. (Eds.), The Amilies and Genera of Vascular Plants Vol. VIII Flowering plants Eudicots, Asterales. Springer, Berlin, Germany. P 364.

Ugulu I, Baslar S, Yorek N, Dogan Y (2009). The investigation and quantitative ethnobotanical evaluation of medicinal plants used around Izmir province, Turkey. J. Med. Plants Res. 3(5): 345-367.

Sosa S, Tubaro A, Kastner U, Glasl S, Jurenitsch J, Della LR (2001) . Topical anti-inflammatory activity of a new germacrane derivative from *Achillea pannonica*. J. Planta Med. 67:654-658.

Simic N, Palic R, Randjelovic V (2005). Composition and antibacterial activity of *Achillea clypeolata* essential oil. Flavour Fragr. J. 20:127–130.

Sokmen A, Sokmen M, Daferera D, Polissiou M, Candan F, Ünlü M, Akpulat HA (2004). The in vitro antioxidant and antimicrobial activities of the essential oil and methanol extracts of *Achillea biebersteini* Afan (Asteraceae). J. Phytother. Res. 18:451–456.

Simoes CMO, Schenkel EP, Gosmann G (1999). Farmacognosia: Da Planta ao Medicamento. Ed. UFRGS, Porto Alegre. P 821.

Afsharypour S, Asgary S, Lockwood GB (1996). Constituents of the essential oil of *Achillea wilhelmsii* from Iran. J. Planta Med. 62:77-8.

Rezaee MB, Jaimand K, Mozaffrian V (2008). Essential oil composition of *Anthemis coelopoda* Boiss. Iran. J. Med. Aromatic Plants 24(3):271-277.

Tohidpour A, Sattari M, Omidbaigi R, Yadegar A, Nazemi J (2010). Antibacterial effect of essential oils from two medicinal plants against Methicillin-resistant *Staphylococcus aureus* (MRSA). J. Phytother. Med. 17:142–145.