African Journal of Pharmacy and Pharmacology Vol. 6(41), pp. 2895-2899, 8 November, 2012
Available online at http://www.academicjournals.org/AJPP
DOI: 10.5897/AJPP12.397
ISSN 1996-0816 © 2012 Academic Journals

Full Length Research Paper

Antimicrobial effects of yarrow (*Achillea millefolium*) essential oils against *Staphylococcus* species

Eilyad Issabeagloo[1]*, Mohammad taghizadieh[2], Babak Abri[3]

[1]Department of Pharmacology, Medical Sciences Faculty, Tabriz branch, Islamic Azad University, Tabriz, Iran.
[2]Department of Pathology, Medical Sciences Faculty, Tabriz branch, Islamic Azad University, Tabriz, Iran.
[3]Department of Surgery, Medical Sciences Faculty, Tabriz branch, Islamic Azad University, Tabriz, Iran.

Accepted 17 October, 2012

***Staphylococcus aureus*, a gram positive, non-motile, catalase and coagulase positive, facultative anaerobe coccus is a common type of bacteria that normally lives on the skin and nasal passages of healthy people. The genus *Achillea* (Asteraceae) was named after the mythological Greek warrior Achilles who used *Achillea* species for healing wounded-soldiers during the Trojan war. The genus Achillea comprises of ~85 species, most of which are endemic to Europe and the Middle East. The aim of this study was to evaluate antimicrobial effects of yarrow (*Achillea millefolium*) essential oils against *Staphylococcus* spp., fourteen clinical isolates of *Staphylococcus* cultured from patients. The disc diffusion method was used for determination of antimicrobial activity of essential oil. Results showed that this inhibitory effect is dose-dependent to wit, by increasing the concentration of the extract in the culture media; reduction in growth was obviously revealed. In conclusion, it can be stated that yarrow essential oils have inhibitory effect against *Staphylococcus* spp.**

Key words: Antimicrobial activity, yarrow (*Achillea millefolium*), essential oils, *Staphylococcus* spp.

INTRODUCTION

Plant substances play a major role in primary health care as therapeutic remedies in many developing countries until today (Zakaria, 1991). Medicinal herbs contain physiologically active principles that over the years have been exploited in traditional medicine for the treatment of various ailments, as they contain anti-microbial properties (Kelmanson et al., 2000; Srinivasan et al., 2001). The antifungal and antibacterial activity exhibited by extract and essential oil of medicinal plants has been demonstrated by several researchers (Delgado et al., 2004; Nasar-Abbas and Kadir Halkman, 2004; Ozcan and Erkmen, 2001; Fazeli et al., 2007) but unfortunately, there are few data related to the antimicrobial activity of extracts obtained from different medicinal plants in Chaharmahal va Bakhtiari (Iran).

Staphylococcus aureus, a gram positive, non-motile, catalase and coagulase positive, facultative anaerobe coccus is a common type of bacteria that normally lives on the skin and nasal passages of healthy people. When it enters the body through a cut or other medical devices, it can cause local or serious infections (Franklin, 1998). Methicillin resistant *S. aureus* (MRSA) has become one of the major causes of nosocomial and community pathogens causing significant morbidity and mortality because there are multi drug resistant pathogens that are resistant to all penicillins, so the option antibiotics for treatment of MRSA infections are limited to antibiotics such as vancomycin, tigecycline, lincozolid and mupirocin (Simor et al., 2007). The patterns of antimicrobial susceptibility of *S. aureus* have been changed worldwide and it has been reported increasingly to be less effective.

Development of mupirocin (dos Santos et al., 2007) and vancomycin (Appelbaum, 2006) microbial resistance

*Corresponding author. E-mail: Dr.e.issabeagloo@gmail.com. Tell: +989144079927.

Table 1. Chemical composition of *A. millefolium* L. essential oil.

Compound	Budding	Beginning of blooming	Intensive blooming	End of blooming
α-Pinene	10.04	0.66	0.64	6.24
camphene	15.37	0.46	0.42	2.34
β-Pinene	16.36	2.13	1.86	38.65
limonene	1.88	1.49	1.39	3.76
γ-Terpinene	6.30	13.07	10.83	3.54
β-Caryophyllene	4.35	7.64	8.07	13.79
α-Humulene	0.42	1.12	0.94	2.34
Germacrene D	1.69	5.64	5.36	10.7
Cadinene	6.13	32.24	28.81	0.68

in MRSA has increased in settings with extensive use of these agents. Microbial resistances to conventional antibiotics and adverse effects of these agents have led to finding new sources as antimicrobial agents. Medicinal plants have a long history of use as traditional medicines for treatment of different kinds of ailments especially for infectious diseases.

The genus *Achillea* (Asteraceae), named after the mythological Greek warrior Achilles, who used *Achillea* species for healing wounded soldiers during the Trojan War (Cheers, 1999). The genus *Achillea* comprises of ~85 species, most of which are endemic to Europe and the Middle East. Turkish flora possesses 42 *Achillea* species and 23 of them are endemic (Duman and Achillea, 2000). These species have some interesting properties and are used in cosmetics, fragrances and agriculture, for example, plant protection (Senatore et al., 2005). Some *Achillea* species have been known to be ethnopharmacologically used in folk remedies for various purposes such as hemorrhoid and wound healing (Baytop, 1999). Herbal teas prepared from some *Achillea* species are very often used in folk medicine as diuretic, for abdominal pain, against diarrhea, flatulence and emmenagogue, moreover for wound healing purposes (Fujita et al., 1995; Honda et al., 1996; Yesilada et al., 1993). *A. biebersteinii* is locally named yarrow, and other species widely used as a folk remedy to treat abdominal pain, wounds and stomachache as well (Sezik et al., 2001; Baytop, 1997).

A.biebersteinii Afan. [Asteraceae, Section: Filipendulinae (D.C.) Boiss] (syn. *A. micrantha*) is a perennial herb, villose, stems erect simple or branched from the base; 30 to 60 cm high; leaves up to 10 cm, oblong-lanceolate in outline, pinnatisect into numerous narrow segments divided into minute linear-lanceolate mucronate lobes; the heads are radiate, in large dense compound corymbs; involucre 4 to 5 mm, oblong-ovoid; flowering period, April to May. Several biological activity studies have been performed on various *Achillea* species, including antibacterial, antioxidant, anti-inflammatory and antispasmodic activities (Karamenderes and Apaydın, 2003; Candan et al., 2003; Al-Hindawi et al., 1989; Skocibusic et al., 2004).

The aim of this study was to evaluate antimicrobial effects of yarrow (*A. millefolium*) essential oils against *Staphylococcus* spp.

MATERIALS AND METHODS

Extract preparation

The aerial parts of *A. millefolium* were air-dried and then ground into fine powder using grinder and stored at room temperature. Dried plant materials were powdered (200 g) and subjected to hydro-distillation (in 2000 ml distilled water) for 4 h, using a Clevenger-type apparatus according to the method recommended in British Pharmacopoeia (1988). The essential oil samples were stored in universal bottles and refrigerated at 4°C prior to use.

Bacterial cultures and preparation of yarrow extracts

Fourteen clinical isolates of Staphylococcus cultured from patients and *S. aureus* ATCC 25923 were used in all experiments. Methicillin resistant *S. aureus* directed was detected on CHROMagarTM MRSA (CHROMagar Paris, France). Bacterial suspensions were made in Brain Heart Infusion (BHI) broth to concentration of approximately 108 CFU/ml using standard routine spectrophometrical method. Subsequent dilutions were prepared from the above suspensions, which were then used in the tests.

Disc diffusion method

The disc diffusion method was used for determination of antimicrobial activity of essential oil. Briefly, using a sterile cotton swab, above microbial suspensions was spread on the Mueller Hinton Agar (MHA) plates. Sterile paper discs (6 mm in diameter) were impregnated with 10, 15, 20 µl of each oil and were placed on the inoculated plates. After remaining at 4°C for 2 h, plates were incubated for 24 h at 37°C. The diameters of the inhibition zones were measured in millimeters. All tests were performed in triplicate (NCCLS, 2009).

RESULTS

Chemical composition of *A. millefolium* L. essential oil is shown in Table 1 (Bimbiraite et al., 2008).

Biochemical test such as catalase, oxidase, coagulase and OF was carried out to proven the genera and data are

Table 2. Results obtained from different biochemical tests.

Genera	Coagulase test	Hemolysis	Pigmented colonies	Mannitol salt agar	Maltose
Staphylococcus aureus	+	+	+	+	+
S. intermedius	+	+	-	d	±
S. hyicus	d	-	-	-	-
S. epidermidis	-	d	-	-	+
S. saprophyticus	-	-	d	d	+
S. aureus ssp. *anaerobious*	+	+	-	0	+
S. capare	-	d	-	d	d
S. gallinarum	-	d	d	+	+
S. arlettae	-	-	+	+	+
S. lentus	-	-	d	+	d
S. equorum	-	d	-	+	d
S. simulans	-	d	-	+	±
S. delphini	0	+	-	+	+
S. chromogenes	-	-	+	d	d

d: 11-89% positive, +: 90% and more positive, -: 90% and more negative, 0: unknown.

Table 3. Anti-staphylococcal activity of yarrow essential oils by disc diffusion method.

Genera	Yarrow		
	Inhibition zone diameter (mm*)		
	10%	20%	30%
S. aureus	8.80	9.12	10.14
S. intermedius	8.78	9.55	10.30
S. hyicus	9.02	9.10	10.37
S. epidermidis	8.87	8.90	9.49
S. saprophyticus	9.10	9.47	9.76
S. aureus ssp. anaerobious	9.32	9.80	9.98
S. capare	7.80	8.20	9.08
S. gallinarum	8.41	9.40	9.73
S. arlettae	9.11	9.71	9.84
S. lentus	8.80	9.09	10.12
S. equorum	9.61	9.64	10.35
S. simulans	8.26	8.70	9.87
S. delphini	7.88	8.65	9.80
S. chromogenes	8.79	8.83	9.54

shown in the Table 2. Inhibitory effect of yarrow extract was determined by different concentrations of this herbal extract and results showed that this inhibitory effect is dose-dependent, to wit, by increasing the concentration of the extract in the culture media, reduction in growth was obviously revealed (Table 3).

DISCUSSION

Essential oils of higher plants have been evaluated against pathogenic microorganisms (bacteria, fungi and yeast) by many other workers (Pandey et al., 2010; Rasooli and Rezaei, 2002; Bussmann and Glenn, 2011) and the results support the findings of the present investigation. An important characteristic of essential oils and their components is their hydrophobicity, which enables them to partition in the lipids of bacterial cell membrane and mitochondria, disturbing the structures and rendering them more permeable as a result of which leakage of ions and other cell contents can then occur (Pirbalouti et al., 2011). Although, a certain amount of leakage from bacterial cells may be tolerable without loss of viability, extensive cell contents loss or the exit of critical molecules and ions will lead to death. It has also been reported that gram-negative strains are less susceptible to essential oils due to the presence of an outer membrane surrounding the cell wall which restricts diffusion of hydrophobic compounds through its lipopolysaccharide covering (Fredj et al., 2007).

The results obtained in the present study revealed that essential oil exhibited variable levels of antibacterial activity against all tested bacterial strains. According to the literature data (Candan et al., 2003; Mimica-Dukic et al., 2004), gram-positive bacteria seemed to be more sensitive to the examined essential oil than gram-negative bacteria. Furthermore, the essential oil obtained from *A. collina* s.l. in the most of the cases exhibited stronger antibacterial activity than *A. pannonica* oil (in some of tested *S. aureus* and Streptococcus strains). This could be due to the presence of high ratio of chamazulene in the essential oil.

On the other hand, stronger bacteriostatic activity of *A. pannonica* was observed on *Streptococcus hyicus* and one strain of *Str. agalactiae* in comparison to *A. collina* oil. This could be explained by notable amounts of 1,8-cineole (40.40%), camphor (11.10%) and borneol (3.22%) in the essential oil. All of the three substances are confirmed as strong antimicrobials on a different range of bacteria (Candan et al., 2003; Tabanca et al., 2001; Tzakou et al., 2001).

Gram-positive bacteria are known to be more susceptible to essential oils than gram-negative bacteria (Inouye et al., 2001). *P. aeruginosa* was least susceptible to the essential oils. The weak antibacterial activity against gram-negative bacteria was ascribed to the presence of their cell wall, lip polysaccharide (Nostro et al., 2000). *B. subtilis* was the most susceptible micro-organism to the rosemary essential oil. Concerning the activity of pure active compounds, the most susceptible bacteria to thymol was *B. subtilis* (23.0 mm) and the most resistant was *P. aeruginosa* (11.5 mm).

The essential oil of *A. distans* W. et K. flower heads was analyzed by GC and Gas chromatography–mass spectrometry (GC-MS). Altogether, 43 components in concentrations more than 0.1% were identified representing 93.5% of the oil composition. The main constituents were 1,8-cineole (16.8%), trans-thujone (9.8%), sabinene (8.2%), borneol (7.5%), beta-pinene (6.5%), and camphor (5.8%). The oil showed moderate activity against *S. aureus* and *Candida albicans*, and weak activity against *Salmonella typhimurium*, *Proteus vulgaris*, and *Escherichia coli* (Konakchiev et al., 2011).

In one study, the screening of the antimicrobial activity of yarrow essential oil was conducted by a disc diffusion test against gram-positive (*B. subtilis*, *B. cereus*, *S. aureus*, *Str. faecalis*), gram-negative (*E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis*) and fungal organisms (*Aspergillus niger*, *A. fumigatus*, *C albicans*). The activity was more pronounced against gram-negative and fungal organisms than against gram-positive bacteria. *A. clavennae* oil was found to possess antimicrobial activity against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and all fungal organisms (Bezić et al., 2003).

Conclusion

From the obtained results, it is obvious that the chemical composition of the essential oil has important impact on both antioxidant and antimicrobial effects of *A. millefolium* obtained from different biological sources. The presence of chamazulene increases the antibacterial activity, whereas the antioxidant and scavenging effects of essential oil are related to some other substances such as camphor and borneol.

REFERENCES

Al-Hindawi MK, Al-Deen IH, Nabi MH, Ismail MA (1989). Anti-inflammatory activity of some Iraqi plants using intact rats. J. Ethnopharmacol. 26(2):163-168.

Appelbaum PC (2006). The emergence of vancomycin-intermediate and vancomycin-resistant Staphylococcus aureus. Clin. Microbiol. Infect. 12(1):16-23.

Baytop T (1997). A Dictionary of vernacular names of wild, publication of the Turkish Language Society. Ankara. 578:163-238.

Baytop T (1999). Therapy with Medicinal Plants in Turkey, Past and Present, Nobel Tıp Kitapevi, Istanbul, Turkey. 2nd ed.

Bezić N, Skocibusić M, Dunkić V, Radonić A (2003). Composition and antimicrobial activity of *Achillea clavennae* L. essential oil. Phytother. Res. 17(9):1037-40.

Bimbiraitė K, Ragažinskienė O, Maruška A, Kornyšova O (2008). Comparison of the chemical composition of four yarrow (*Achillea millefolium* L.) morphotypes. Biologija 54(3):208-212.

British Pharmacopoeia (1988). Her Majesty's Stationery Office, (HMSO), London. Vol. 2, pp. 37-138.

Bussmann RW, Glenn A (2011). Medicinal plants used in Northern Peru for the treatment of bacterial and fungal infections and inflammation symptoms. J. Med. Plants Res. 5(8):1297-1304.

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(2-3):215-20.

Cheers G (1999). Botanica: the illustrated A-Z of over 10,000 garden plants and how to cultivate them. Könemann, Hong Kong p 1020.

Delgado B, Palop A, Fernandez PS, Periago PM (2004). Combined effect of thymol and cymene to control the growth of *Bacillus cereus* vegetative cells. Eur. Food Res. Technol. 218:188-193.

Dos Santos KRN, de Souza FL, Filho PPG (2007). Emergence of high-level mupirocin resistance in methicillin-resistant *Staphylococcus aureus* isolated from Brazilian university hospitals. Infect. Control Hosp. Epidemiol. 17:813-816.

Duman H, Achillea L (2000). Flora of Turkey and the East Aegean Islands. In: Guner A, Ozhatay N, Ekim T, Baser KHC (eds). Edinburgh University Press, Edinburgh. pp. 158-159.

Fazeli MR, Amin GHR, Ahmadian AMM, Ashtiani H, Jamalifar H, Samadi N (2007). Antimicrobial activities of Iranian sumac and avishan-e-shirazi (*Zataria multiflora*) against some food-borne bacteria. Food Control 18:646-649.

Franklin DL (1998). *Staphylococcus aureus* infection. New Engl. J. Med. 339(8):520-532.

Fredj MBH, Marzouk B, Chraief I, Boukef K, Marzouk Z (2007). Analysis of Tunisian *Ruta graveolens* L. oils from Jemmel. J. Food Agric. Environ. 5(1):52-55.

Fujita T, Sezik E, Tabata M (1995). Traditional medicine in Turkey VII. Folk medicine in middle and west Black Sea regions. Econ. Bot. 49(4):406-422.

Honda G, Yesilada E, Tabata M, Sezik E, Fujita T, Takeda Y, Takaishi Y, Tanaka T (1996). Traditional medicine in Turkey VI. Folk medicine in West Anatolia: Afyon, Kütahya, Denizli, Muğla, Aydin provinces. J. Ethnopharmacol. 53(2):75-87.

Inouye S, Takizawa T, Yamaguchi H (2001). Antibacterial activity of essential oils and their major constituents against respiratory tract pathogens by gaseous contact. J. Antimicrob. Chemother. 47(5):565-73.

Karamenderes C, Apaydın S (2003). Antispasmodic effect of *Achillea nobilis* L. subsp. *sipylea* (O. Schwarz) Bassler on rat isolated duodenum. J. Ethnopharmacol. 84(2-3):175-179.

Kelmanson JE, Jager AK, Van Staden J (2000). Zulu medicinal plants with antibacterial activity. J. Ethnopharmacol. 69:241-246.

Konakchiev A, Todorova M, Mikhova B, Vitkova A, Najdenski H (2011). Composition and antimicrobial activity of *Achillea distans* essential oil. Nat. Prod. Commun. 6(6):905-6.

Mimica-Dukic N, Bozin B, Sokovic M, Simin N (2004). Antimicrobial and antioxidant activities of *Melissa officinalis* L. (Lamiaceae) essential oil. J. Agric. Food Chem. 52(9):2485-9.

Nasar-Abbas SM, Kadir HA (2004). Antimicrobial effect of water extract of sumac (*Rhus coriaria* L.) on the growth of some food borne bacteria including pathogens. Int. J. Food Microb. 97:63-69.

NCCLS (2009). Methods for dilution of Antimicrobial susceptibility tests for bacteria that grow aerobically. Approved Standard M7-A8, 8th ed, Wayne, Pennsylvania (PA).

Nostro A, Germano MP, D´Angelo V, Marino A, Cannatelli MA (2000). Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Lett. Appl. Microbiol. 30(5):379-84.

Ozcan M, Erkmen O (2001). Antimicrobial activity of the essential oils of Turkish plant spices. Eur. Food Res. Technol. 212:658-660.

Pandey RR, Dubey RC, Saini S (2010). Phytochemical and antimicrobial studies on essential oils of some aromatic plants. Afr. J. Biotechnol. 9(28):4364-4368.

Pirbalouti AG, Hamedi B, Malek PF, Rahimi E, Nasri NR (2011). Inhibitory activity of Iranian endemic medicinal plants against *Vibrio parahaemolyticus* and *Vibrio harveyi*. J. Med. Plants Res. 5(32):7049-7053.

Rasooli I, Rezaei MB (2002). Bioactivity and chemical properties of essential oils from *Zataria multiflora* Boiss. and *Mentha longifolia* (L.) Huds. J. Essential Oil Res. 14(2):141-146.

Senatore F, Napolitano F, Arnold NA, Bruno M, Herz W (2005). Composition and antimicrobial activity of the essential oil of *Achillea falcata* L. (Asteraceae). Flavour Fragr. J. 20(3):291-294.

Sezik E, Yesilada E, Honda G, Takaishi Y, Takeda Y, Tanaka T (2001). Traditional medicine in Turkey X. Folk medicine in Central Anatolia. J. Ethnopharmacol. 75(2-3):95-115.

Simor AE, Stuart TL, Louie L, Watt C, Ofner-Agostini M, Gravel D, Mulvey M, Loeb M, McGeer A, Bryce E, Matlow A (2007). Mupirocin-Resistant, Methicillin-Resistant *Staphylococcus aureus* Strains in Canadian Hospitals. Antimicrob. Agents Chemother. 51(11):3880-3886.

Skocibusic M, Bezic N, Dunkic V, Radonic A (2004). Antibacterial activity of Achillea clavennae essential oil against respiratory tract pathogens. Fitoterapia 75(7-8):733-736.

Srinivasan D, Sangeetha N, Suresh T, Perumalsamy PL (2001). Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. J. Ethnopharmacol. 74:217-220.

Tabanca N, Kirimer N, Demirci B, Demirci F, Başer KH (2001). Composition and antimicrobial activity of the essential oils of *Micromeria cristata* subsp. *phrygia* and the enantiomeric distribution of borneol. J. Agric. Food Chem. 49(9):4300-3.

Tzakou O, Pitarokili D, Chinou IB, Harvala C (2001). Composition and antimicrobial activity of the essential oil of *Salvia ringens*. Planta Med. 67(1):81-3.

Yesilada E, Honda G, Sezik E, Tabata M, Goto K, Ikeshiro Y (1993). Traditional medicine in Turkey IV. Folk medicine in the Mediterranean subdivision. J. Ethnopharmacol. 39(1):31-38.

Zakaria M (1991). Isolation and characterization of active compounds from medicinal plants. Asia Pac. J. Pharmacol. 6:15-20.