Journal of Animal and Veterinary Advances 7 (4): 508-511, 2008
ISSN: 1680-5593

In vitro Assessment of Antimicrobial Efficacy of Alcoholic Extract of Achillea Millefolium in Comparison with Penicillin Derivatives

[1]Hossien Tajik, [2]Farnood Shokouhi Sabet Jalali, [3]Abdollah Sobhani,
[1]Yasser Shahbazi and [1]Mahsa Soleiman Zadeh
[1]Department of Food Hygiene, [2]Department of Clinical Sciences,
Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
[3]Department of Animal Sciences, Faculty of Agriculture,
Mohaghegh Ardabili University, Ardabil, Iran

Abstract: *Achillea millefolium* (Yarrow) has been used as a spice and medicinal plant in many ancient cultures from olden times. Today, biochemical investigations have shown different bioactive components responsible to medicinal and therapeutic properties of yarrow, in particular antimicrobial effect of it. Aim of this study is *in vitro* assessment of potential of inhibitory function of alcoholic extract of *Achillea*. millefolium on some microorganisms and comparison antimicrobial activity of yarrow with some antibiotics from penicillin family. The plants were used in this investigation, collected from Urmia region. The alcoholic extract of aerial parts of *Achillea millefolium* has been tested for antimicrobial activity in a disk diffusion assay. Also, microorganisms were used in this examination were divided in 2 categories: Control (*Staphylococcus aureus*, *Salmonella enteritidis, Escherichia coli*) and clinical isolated microorganisms (*Staphylococcus aureus*, Sterptococos Pneumoniae, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, Enterobacter aerogenes, Proteus mirabilis) and 2 fungi (*Aspergillus niger* and *Candida albicans*). According to results of this study, the most sensitive organism to extract of yarrow was Staphylococcus aureus ($p<0.05$). The antibacterial activity of the plant was lesser to Ampicillin and greater or similar to other penicillin derivatives. With attention to our finding and other related reports in this field, it could be concluded alcoholic extract of yarrow has considerable antimicrobial effect on control and wound pathogen microorganisms. In spite of it, before application of the extract as antimicrobial agent, it must be more evaluated *in vivo* and clinically.

Key words: Yarrow, alcoholic extract, antimicrobial, wound, penicillin

INTRODUCTION

Achillea millefolium L. (Asteraceae), popularly known as yarrow, is a widely distributed medicinal plant that has been used for over 3000 years (Mitich, 1990). Popular indications of this specie include treatment of wounds, hemorrhages, headaches, inflammation, pain, spasmodic diseases, flatulence and dyspepsia (Correia, 1974; Chandler *et al*., 1982; Blumenthal *et al*., 2000). Some of these reputed folk effects have been determined showing the potential medicinal use of the plant. The medicinal properties of *Achillea millefolium* are worldwide recognized and the plant is included in the national *Pharmacopoeias* of countries such as Germany, Czech Republic, France and Switzerland (Mitich, 1990; Bradley, 1992; Alonso, 1998; Blumenthal *et al*., 2000). *Achillea millefolium* seems to have originated in European folk medicine and from this origin, the herb has been spread to east (Perry, 1980).

In Iran, *Achillea millefolium* is included in the list of the popular medicinal plants of the Iranian traditional medicine supported by the Ministry of Health, Therapy and Medical Teaching (MHTMT). Preparations of *Achillea millefolium* have been shown to have anti-inflammatory (Goldberg *et al*., 1969; Tunon *et al*., 1995), antitumor (Tozyo *et al*., 1994), antioxidant (Candan *et al*., 2003) and liver protective activities (Gagdoli and Mishra, 1995; Lin *et al*., 2002). Studies were carried out on yarrow showed essential oil, alcoholic extract and water-soluble of this plant have broad spectrum antimicrobial properties (Barel, 1991). Linalool, found at up to 26% of the essential oil fraction in hexaploids, which are the most common

Corresponding Author: Hossien Tajik, Department of Food Hygiene, College of Veterinary Medicine, P.O. Box 57155-1177, Urmia University, Urmia, Iran

subsp. of *A. millefolium*, has been shown to inhibit 17 types of bacteria and 10 fungi (Pattnaik, 1997). Yarrow is also used for disorders of the respiratory, digestive, hepatobiliary, cardiovascular, urinary and reproductive systems (Hoffmann, 1990; Bradley, 1992; Mills, 1994; Bown, 1995; Blumenthal *et al*., 2000).

The aim of the present study was to evaluate antimicrobial activity of *Achillea millefolium* in form of alcoholic extract against some microorganisms (control and wound pathogen bacteria) and in comparison with penicillin family antibiotics.

MATERIALS AND METHODS

Collection of plant material: Aerial parts of yarrow were collected during the flowering period and the vegetative phase (13-15th July 2007), from Urmia area in west north of Iran. Taxonomic identity of the plant was confirmed by comparing collected voucher specimen with those of known identity in the herbarium of the department of botanical sciences, investigation institute of agriculture organization of Iran.

Preparation of the extract: The extract for the antimicrobial assay was prepared by maceration (in a dark place during 5 days with occasional shaking) of 5 g of air-dried and finely ground plant material with 100 mL of the solvent obtained by mixing equal volumes of ether, hexane and methanol. The resulting extract after the removal of the solvent mixture on a rotary evaporator was obtained in the following yields (%, w w^{-1}): 8.84. The extract of *Achillea millefolium* utilized for the composition investigation was obtained by the same procedure only with 10 g of starting plant material and this afforded 1.2 g of dry extract.

Isolation of constituents of *Achillea millefolium* extract: The extract was subjected to silica gel column chromatography (CC). On elution with hexane-ethyl acetate in a gradient in order to increase polarity (from 0-100% EtOAc and afterwards by washing the column with methanol) 9 fractions were obtained (Table 1). First 4 fractions were analyzed by GC-MS. Fractions 5-9 were first methylated on treatment with trimethylsilyl-diazomethane in methanol and the usual work up which was followed with a GC-MS analysis (Table 1).

Table 1: The fraction composition of the *Achillea millefolium* extract

Fraction	Weight (mg)	(%)	Composition	Methods of identification
1	38.4	3.2	n-Alkanes C15-C29 (maximum at C23, 22.3%)	GC-MS
2	170.6	14.2	1,8-Cineole, camphor and caryophyllene oxide, n-alkanes C15-C29 (maximum at C29, 18.4%)	GC-MS
3	41.3	3.4	4-Terpineol, eugenol, spathulenol, methyl ester of 11,14,17-eicosatrienoic acid	GC-MS GC-MS
4	19.9	1.6	Phytol, octa-, nona- and eicosanol	GC-MS
9	25.3	2.1	Fatty acids, even numbered C14-C24, C16(48.3%) and C18:2 (35.6%)	(after methylation)

Test microorganisms: The *in vitro* antimicrobial activities of the extract of *Achillea millefolium* were tested against a panel of laboratory control strains: Gram-positive: *Staphylococcus aureus* (ATCC 25923), Gram-negative: *Salmonella typhimurium* (ATCC 1730) and *Escherichia coli* (ATCC 25922) were obtained from Department of Food Hygiene of the Faculty of Veterinary Medicine of Urmia University, Urmia, Iran. Using Stokes (1993) method, some multi-resistant organisms isolated from clinical wound cases (referred to Teaching Hospital of the Faculty of Veterinary Medicine of Urmia University) were subjected to sensitivity test using the extract of yarrow at their antimicrobial activity end points.

Evaluation of antimicrobial activity: The agar disc diffusion method was employed for the determination of antimicrobial activities o f the extract of yarrow. Briefly, a suspension of the tested microorganism (0.1 mL of 10^8 cells mL^{-1}) was spread on the solid media plates. Filter paper discs (6 mm in diameter) were impregnated with 50 μL of the extract and for comparative evaluation of antimicrobial activities, the standard antibiotic discs of penicillin derivatives: Penicillin (10 IU/disc), Ampicillin (10 mcg/disc), Amoxicillin (25 mcg/disc), Cloxacillin (1 mcg/disc), Carbenicillin (100 mcg/disc) (All standard antibiotic discs were prepared from Padtan Teb, Tehran, Iran), placed on the inoculated plates, they were incubated at 37°C for 24 h for bacteria and at 30°C for 48 h for the yeasts. The diameters of the inhibition zones were measured in millimeters. The details of this procedure are described in the recent reference Quang *et al*. (2005). All the tests were performed in duplicate.

RESULTS AND DISCUSSION

The *in vitro* antimicrobial tests of the alcoholic extract of *A. millefolium* resulted in a range of growth inhibition pattern against control and wound pathogenic microorganisms (Table 1 and 2).

Based on results obtained from disc diffusion method, *Staphylococcus aureus* is the most sensitive microorganism in both groups (control and wound pathogen organisms). Follow this organism, in control group, *S. typhimorium* and *Escherichia coli* were

Table 2: Antimicrobial activity of the alcoholic of *Achillea millefolium* against wound pathogens microorganisms by using agar disc diffusion method

Microorganisms Plant specie	*A .millefolium*
Staphylococcus aureus	20.00±0.12
Sterptococos. pneumoniae	18.12±0.42
Enterobacter aerogenes	11.02±0.40
Escherichia coli	12.20±0.24
Klebsiella pneumoniae	8.00±0.14
Proteus mirabilis	8.02±0.02
Pseudomonas aeruginosa	8.00±0.42
Candida albicans	60.0±0.04
Aspergilluos niger	60.0±0.02

Table 3: Antimicrobial activity of the alcoholic of *Achillea millefolium* against control microorganisms by using agar disc diffusion method

Plant specie	*A.millefolium*	The DD of antibiotics				
Microorganisms	DD*	*Penicillin*	*Ampicillin*	*Amoxicillin*	*Cloxacillin*	*Carbenicillin*
Staphylococcus aureus	20.04±0.22	24.37±0.12	27.05±0.19	25.24±0.09	27.78±0.14	0.00
S.typhimorium	17.24±0.12	9.46±0.56	17.35±0.42	14.79±0.5	0.00	17.11±0.11
Escherichia coli	12.02±0.42	10.53±0.22	11.51±0.19	10.95±0.13	0.00	11.81±0.11

sensitive, respectively. But in wound pathogen group, *S. pneumoniae*, *Escherichia coli* and *Enterobacter aerogenes* were sensitive test microorganisms against the extract of *A. millefolium*, respectively. The remaining microorganisms were found to be resistant against the extract tested exhibiting weak inhibition zones (<10 mm) (Table 3).

The antibacterial activity of *A. millefolium* was lesser to Ampicillin and greater to other penicillin's derivatives (Penicillin, Amoxicillin and Carbenicillin), especially in the cases of 2 g negative bacteria of control group (*S.typhimorium* and *Escherichia coli*).

In this study, the alcoholic extract of yarrow has antibacterial efficacy against all tested microorganisms except against some microorganisems in the important wound pathogens such as Klebsiella *pneumoniae* which turned out to be the most resistant of the microorganisms. The activity of *Achillea millefolium* was, as published before for methanol extract (Candan *et al*., 2003), lower and no bactericidal was observed at all against *Klebsiella pneumoniae*.

The common observation for the inhibitory effect of the essential oil of yarrow was against the Gram-negative bacteria more susceptible than the Gram-positive (Smith-Palmer *et al*., 1998). But our findings were not agreed with above report. In the present study, the Gram- positive bacteria are more susceptible than the Gram-negative microorganisms to alcoholic extract of *A. millefolium.*

The extracts of *Achillea genus* can be divided into different groups according to the antimicrobial nature of the extracts and attributed to the presence of 2 distinct types of secondary metabolites present in the extracts.

One of these groups is consisted of *Achillea millefolium* (Lyss *et al*., 2000), which have most probably sesquiterpene lactones as active principles (along with the all occurring flavonoids in the genus *Achillea*). Rupicolins and respected derivates have not been tested yet for antimicrobial activity, but their antiphlogistic activity was demonstrated (Zitterl-Eglseer *et al*., 1991). It is believed that the methylene lactone moiety of the molecule is responsible for the activity as shown in the case of methyl lactones from *Achillea millefolium* and *Achillea atrata* (Aljan *et al*., 1999).

Also, report concerning the antimicrobial activities of the essential oils from *A. millefolium*, terpinen was concluded as one of the compounds responsible for bacteriosidic effect against several microorganisms (Barel *et al*., 1991). Although in minor percentages this compound was identified in GC/MS analysis of the oils extracted from *A. millefolium* and together with the major compounds identified, e.g., camphor and their derivatives, eucalyptol (1, 8-cineol) and borneol can be considered as the antimicrobial constituents of the oils of this medicinal plant (Candan *et al*., 2003).

CONCLUSION

In conclusion, our observations confirm that alcoholic extract of *Achillea millefolium* possess strong antimicrobial activity against to the control and wound pathogen microorganisms and its property is the greater or similar to the tested penicillin's derivatives. In spite of it, before application of the extract as antimicrobial agent, it must be more evaluated *in vivo* and clinically.

ACKNOWLEDGEMENT

The authors wish to thank the Vice Chancellor for Research of Faculty of Veterinary Medicine of Urmia University for the financial support of this study. The

isolation of the constituents of *Achillea millefolium* extract was conducted in the Biochemical Laboratory of the Institute of Medicinal plants (Tehran, Iran).

REFERENCES

Aljan˘ci´c, I., V. Vajs, N. Menkovi´c, I. Karad˘zi´c, N. Jurani´c, S. Milosavljevi´c and S. Macura, 1999. Flavones and sesquiterpene lactones from *Achillea atrata* subsp. *multifida*: Antimicrobial activity. J. Natural Prod., 62: 909-911.

Alonso, J.R., 1998. Milenrama. In: Tratado de Fitomedicina: Bases cl´ınicas yfarmacol´ogicas. Isis, Buenos Aires, pp: 725-729.

Barel, S., R. Segal and J. Yashphe, 1991. The antimicrobial activity of the essential oil from *Achillea fragrantissima*. J. Ethnopharmacol., 33: 187-191.

Bown, D., 1995. Encyclopaedia of Herbs and their Uses. Dorling Kindersley, London, pp: 101-105.

Bradley, P.R., 1992. British herbal compendium, British Herbal Med. Assoc. Bournemouth, 1: 190-191.

Blumenthal, M., W.R. Busse, A. Goldberg, J. Gruenwald, T. Hall, C.W. Riggins and R.S. Rister, 2000. Yarrow. In: Herbal medicine expanded commission emonographs. Integrative Med. Commun. Boston, pp: 419-423.

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

Chandler, R.F., S.N. Hooper, D.L. Hooper, W.D. Jamieson, C.G. Flinn and L.M. Safe, 1982. Herbal remedies of the maritime Indians: Sterols and triterpenes of *Achillea millefolium* L. (yarrow). J. Pharm. Sci., 71 (6): 690-693.

Correia, P.M., 1974. Dicion´ario de plantas ´uteis do Brasil e das ex´oticas cultivadas. Rio de Janeiro: Minist´erio da Agricultura e Instituto Brasileiro de Desenvolvimento Florestal, 5: 687.

Gagdoli, C. and S.H. Mishra, 1995. Preliminary screening of *Achillea millefolium, Cichorium intybus* and *Capparis spinosa* for antihepatotoxic activity. Fitoterapia, 66: 319-323.

Goldberg, A.S., E.C. Mueller, E. Eigen and S.J. Desalva, 1969. Isolation of the anti-inflammatory principles of *Achillea millefolium* (Compositae). J. Pharm. Sci., 58: 938-941.

Hoffmann, D., 1990. The New Holistic Herbal, Element, Dorset, Great Britain, pp: 94-98.

Lin, L.T., L.T. Liu, L.C. Chiang and C.C. Lin, 2002. *In vitro* anti-hepatoma activity of 15 natural medicines from Canada. Phytotherap. Res., 16: 440-444.

Lyss, G., S. Glasl, J. Jurenitsch, H.L. Pahl and I. Merfort, 2000. A sesquiterpene and sesquiterpene lactones from the *Achillea millefolium* group possess antiinflammatory properties but do not inhibit the transcription factor NF-_B. Pharm. Pharmacol. Lett., 10: 13-15.

Mills, S., 1994. The Complete Guide to Modern Herbalism. Thorsons, Great Britain, pp: 55-59.

Mitich, L.W., 1990. Intriguing world of weeds: Yarrow- the herb of achilles. Weed Technol., 4: 451-453.

Quang, D.N., T. Hashimoto, J. Fournier, M. Stadler, N. Radulovi´c and Y. Asakawa, 2005. Sassafrins A-D, new antimicrobial azaphilones from the fungus *Creosphaeria sassafras*. Tetrahedron, 61: 1743-1748.

Pattnaik, S., V.R. Subramanyam and M. Bapaji, 1997. Antibacterial and antifungal activity of ten essential oils *in vitro*. Microbios, 89: 39-46.

Perry, L.M., 1980. Medicinal Plants of East and Southeast Asia. The MIT Press, Cambridge, United Kingdom, pp: 110-113.

Smith-Palmer, A., J. Stewart and L. Fyfe, 1998. Antimicrobial properties of plant essential oils and essences against 5 important food-born pathogens. Lett. Applied Microbiol., 26: 118-122.

Stokes, E.S., G.I. Ridway and M.W. Wren, 1993. Clinical Microbiology. 7th Arnold London, pp: 86-87.

Tozyo, T., Y. Yoshimura, K. Sakurai, N. Uchida, Y. Takeda, H. Nakai and H. Ishii, 1994. Novel antitumor sesquiterpenoids in *Achillea millefolium*. Chem. Pharm. Bull., 42: 1096-1100.

Tunon, H., C. Olavsdotter and L. Bohlin, 1995. Evaluation of anti-inflammatory activity of some Swedish medicinal plants. Inhibition of prostaglandin biosynthesis and PAF-induced exocytosis. J. Ethnopharmacol., 48: 61-76.

Zitterl-Eglseer, K., J. Jurenitsch, S. Korhammer, E. Haslinger, S. Sosa, R. Della Loggia, W. Kubelka and C. Franz, 1991. Sesquiterpene lactones of *Achillea setacea* with antiphlogistic activity. Planta Medica, 57: 444-446.