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Full Length Research Paper

Ethnobotany and antimicrobial activity of medicinal plants of Bakhtiari Zagross mountains, Iran

A. Ghasemi Pirbalouti*, F. Malekpoor and B. Hamedi

Shahrekord Branch, Islamic Azad University, Department of Medicinal Plants, Researches Centre of Medicinal Plants and Ethno-veterinary, P. O. Box: 166,Shahrekord, Iran.

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The major aims of this study was to determine ethnobotany and antimicrobial activity of sixteen medicinal, aromatic and nutraceutical plants from high altitude of Bakhtiari Zagross mountains, which are Iranian medicinal plants. Antimicrobial activities of extract of sixteen Iranian folklore plants including, *Heracleum lasiopetalum* Boiss. (Apiaceae), *Satureja bachtiarica* Bunge. (Lamiacae), *Thymus daenensis* Celak. (Lamiacae), *Ziziphora teniur* L. (Lamiacae), *Echiophora platyloba* L. (Apiaceae), *Dracocephalum multicaule* Benth. (Lamiacae), *Kelussia odoratissima* Mozff. (Apiaceae), *Mentha longifolia* Hudson. (Lamiacae), *Achillea kellalensis* Boiss. (Asteraceae), *Stachys lavandulifolia* Vahl. (Lamiacae), *Hypericum scabrum* L. (Hypericaceae), *Quercus brantii* Lindley. (Fagaceae), *Myrtus communis* L. (Myrtaceae), *Pistachia atlantica* Desf. (Anacardiaceae), *Arnebia euchroma* (Royle.) Johnston. (Boraginaceae) and *Salvia hydrangea* DC. (Lamiacae) were investigated against strains of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli* O157:H7, *Yersinia enterocolitica*, *Bacillus cereus, Listeria monocytogenes, Campylobacter coli, Campylobacter jejuni* and *Candida albicans* by agar disc diffusion and serial dilution assays. The results of the study reveal that some of species play an important role in primary healthcare system of these tribal communities. Some of the medicinal plants showed relatively high antimicrobial activity against all the tested bacteria and fungi. In conclusion, it can be said that the extract and essential oil of some of the medicinal plants from high altitude of Bakhtiari Zagross Mountains could be used as natural antibacterial agents in the food preservation and human health.

Key words: Medicinal plants, ethnobotany, antimicrobial activity, high altitude, Zagross mountains.

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 (Aktug and Karapinar, 1986; Arora and Kaur, 1999; Delgado et al., 2004; El-Khateib and Abd El-Rahman, 1987; 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). Numerous Iranian folklore herbs for example: *Heracleum lasiopetalum* Boiss., *Satureja bachtiarica* Bunge., *Thymus daenensis* Celak., *Ziziphora teniur* L., *Echiophora platyloba* L., *Dracocephalum multicaule* Benth., *Kelussia odoratissima* Mozff., *Mentha longifolia* Hudson., *Achillea kellalensis* Boiss., *Stachys lavandulifolia* Vahl., *Hypericum scabrum* L., *Quercus branti* Lindley., *Myrtus communis* L., *Pistachia atlantica* Desf., *Arnebia euchroma* (Royle.) Johnston. and *Salvia hydrangea* DC. have been utilized as traditional medicines by the indigenous people of Chaharmahal va Bakhtiari, Southwest Iran (Ghasemi Pirbalouti, 2009). Hence, it is necessary to establish the scientific basis for the therapeutic actions of traditional plant medicines

*Corresponding author. E-mail: ghasemi@iaushk.ac.ir.

as these may serve as the source for the development of more effective drugs. In this study, it was aimed to determine antimicrobial activity of ethanol extract and essential oils of 16 plant species which are Iranian endemic plants.

MATERIALS AND METHODS

An ethnobotanical survey was conducted in Chaharmahal va Bakhtiari Province, South-West of Iran. The study area is located between latitude 31º 10´ to 32º 45´ N and longitude 49º 29´ to 52 º 34´ E. It occupies an area of 10893 km². The elevation range is between 1009 m in the south to 4250 m in the west districts. The survey was conducted by interviewing traditional healers in each locality using the local language. Each interview followed a semi-structured questionnaire designed to obtain the following information: Scientific and local plant names; habit; plant parts used; uses/ailments treated. The information was collected from 55 persons (20 men and 35 women) in 15 villages, mostly villages of the southern and western parts of the area. The plants were collected from mountain areas of Zagross, Chaharmahal va Bakhtiari district, during 2008 to 2010. Their identity was confirmed and voucher specimens were deposited at the Researches Centre of Medicinal and Aromatic Plants, Islamic Azad University, Shahrekord Branch, Iran. Dried plant material were powered (200g) and subjected to hydro-distillation (2000 ml distillated water) for 4 h using a Clevenger- type apparatus according to the method recommended in British pharmacopoeia (British Pharmacopoeia, 1988).

Air-dried and powdered Leaves and flowers (100 g) were macerated at room temperature with 1 L of ethanol: water (80:20) for 24 h. The extractions continued two times and then were concentrated in a rotary evaporator under reduced pressure (Model Zirbus 302®, Italy). The extract samples were stored in universal bottles and refrigerated at 4°C prior to use.

The antimicrobial activity of 16 Iranian medicinal plants was determined against *Staphylococcus aureus*, *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli* O157: H 7*, Yersinia enterocolitica, Bacillus cereus, Listeria monocytogenes, Campylobacter coli, Campylobacter jejuni* and *Candida albicans*. This microorganism was obtained from Department microbiology of Islamic Azad University, Shahrekord Branch, Iran. A final bacterial suspension containing 10^7 CFU/ml was made from the flask broth culture. Subsequent dilutions were made from the above suspension, which were then used in tests.

The disc diffusion method of Iennette (1985) was used with some modification to determinate rate of inhibition growth of bacteria and fungi by plant extract and essential oil. BHI agar (Merck, Germany) was used to prepare the culture medium and autoclaved at 121°C for 15 min. Briefly, plates (8-cm diameter) were prepared with 10 ml agar inoculated with 1 ml bacterial suspension. Sterile paper discs (6 mm in diameter) were impregnated with 60 µl of dilutions of known extract concentrations (100 µg/disc) and incubated at 35°C for 72 h. The extracts were dissolved in dimethyl sulfoxid (DMSO, 15 µl) before the test for antimicrobial activity. Antimicrobial growth inhibition was determined as the diameter of the inhibition zones around the discs (mm). The growth inhibition diameter was an average of three measurements, taken at three different directions. All tests were performed in triplicate.

RESULTS

The results of the ethnobotanical survey are presented in Table 1. The Growth inhibition value of extract and essential oil on bacteria and fungi strains are shown in Table 2. Most of the extracts and essential oils showed relatively antimicrobial activity against the tested bacteria and fungi with the diameter of inhibition zone ranging between 7 and 30 mm. Antimicrobial activities of extract and essential oil of plants varied related to the test organism. There were significant differences in the antibacterial activities of plant extract and essential oils. Among the plants tested essential oil of *Satureja bachtiarica* and *Thymus daenensis* showed the best antimicrobial activity.

Subsequent experiments were conducted to determine minimal inhibitory concentration and minimal bactericidal and fungicidal concentration of all selected plant extracts and essential oils. The MIC values for active extract and essential oil ranging between 0.039 and 10 mg/ml. The most active of the concentrations was 10 mg/ml concentration inhibiting completely the growth of bacteria and fungi. The results obtained appeared to confirm the antibacterial potential of the plants investigated.

DISCUSSION

Antimicrobial activities of extract and essential oil of plants varied related to the test organisms. The results showed that bacteria (*E. coli*, *L. monocytogenes* and *B. cereus*) were more sensitive than bacteria (*K. pneumonia, E. coli* O157: H 7*, Y. enterocolitica, C. coli,* and *C. jejuni*). In this experimental, *E. coli* was the most sensitive, while *E. coli* O157: H 7 was the most resistant. Shan et al. (2007) reported that Gram-positive bacteria (*L. monocytogenes*, *S. aureus* and *B. cereus*) were generally more sensitive to the tested extracts than Gram-negative (*E. coli* and *Salmonella anatum*).

A possible explanation for these observations may lie in the significant differences in the outer layers of Gram-negative and Gram-positive bacteria. Gram-negative bacteria possess an outer membrane and a unique periplasmic space not found in Gram-positive bacteria (Duffy and Power, 2001). The resistance of Gram-negative bacteria towards antibacterial substances is related to the hydrophilic surface of their outer membrane which is rich in lipopolysaccharide molecules, presenting a barrier to the penetration of numerous antibiotic molecules and is also associated with the enzymes in the periplasmic space, which are capable of breaking down the molecules introduced from outside (Nikaido, 1994; Gao et al., 1999). Gram-positive bacteria do not have such an outer membrane and cell wall structure. Antibacterial substances can easily destroy the bacterial cell wall and cytoplasmic membrane and result in a leakage of the cytoplasm and its coagulation (Kalemba and Kunicka, 2003).

Some studies claim that the phenolic compounds present in spices and herbs might also play a major role

Table 1. Ethnobotany of Medicinal plants from high altitude of Bakhtiari Zagross mountains, Iran.

Scientific name	Family name	Local name	Habit*	Parts used	Uses/ailments treated
Achillea kellalensis Boiss. and Hausskn.	Asteraceae	Golberenjaz	H	flowers	Wound, carminative, indigestion
Arnebia euchroma (Royle.) Johnston.	Boraginaceae	Sorya	H	rhizome, root	Burn wound, anti-eczema, antimicrobial, anti- inflammatory
Dracocephalum multicaule Montbr and Auch.	Lamiaceae	Zarrin giah, Zeravi	H	leaves, flowers	Sedative, analgesia, inflammatory, anti-bacterial, anti-septic, foot pain
Echinophora platyloba DC.	Apiaceae	Khosharizeh	S	aerial plant	Anti fungal, spice and culinary
Heracleum lasiopetalum Boiss	Apiaceae	Goolpar, Kereson	H	fruit	Anti-septic, spice and condiment
Hypericum scabrum L.	Hypericaceae	Golraye dayhimi	H	flowers	Green tea, sedative, headache, analgesic
Kelussia odoratissima Mozaff.	Apiaceae	Kelus	H	leaves	Edible as vegetable, flavoring, indigestion, rheumatism, sedative
Mentha longifolia (L.) Hudson.	Lamiaceae	Pooneh, Pineh	H	leaves, flowers	Edible as vegetable, flavoring, indigestion, cough
Myrtus communis L.	Myrtaceae	Mort	T	leaves	Skin discords, digestive discords, astringent, good hair condition, bronchodilatotor
Pistachia atlanta Desf.	Anacardiaceae	Baneh,	T	resin, fruit	Indigestion, tonic, toothache, astringent
Quercus branti Lindley.	*Fagaceae*	Balout	T	fruit	Anti-ulcer, indigestion
Salvia hydrangea DC.	Lamiaceae	Gool ouroneh	H	leaves, flowers	Cough, emollient, sore throat, antibacterial
Satureja bachtiarica Bung.	Lamiaceae	Marzeh Koohi	H	leaves, flowers	Edible as vegetable, flavoring, indigestion, cough, anti-bacterial
Thymus daenensis Celak.	Lamiaceae	Oushon	H	leaves, flowers	Green tea, spice, culinary, cough, anti-bacterial, carminative
Stachys lavandulifolia Vahl.	Lamiaceae	Lolopashmak, Chaye Koohi	H	leaves, flowers	Green tea, anti-bacterial, skin diseases, menorrhagia
Ziziphora tenuior L.	Lamiaceae	Kakouti	H	leaves, flowers	Green tea, spice, culinary, anti-bacterial, carminative, anti-asthmatic

*Habit: T: Tree, H: Herb.

in their antimicrobial effects (Hara-Kudo et al., 2004). There has been no large scale systematic investigation of the relationship between bacterial inhibition and total phenolic content of spices and herbs. Previous studies (Shan et al., 2005) showed that a highly positive linear relationship exists between antioxidant activity and total phenolic content in some spices and herbs. Many herb and spice extracts for example *T. daenensis* and *S. bachtiarica* contained high levels of phenolics and exhibited antibacterial activity against food-borne pathogens. Previous studies (Rasooli et al., 2006) on the antimicrobial activity of the essential oils of some *Thymus* spp., most of them possessing large quantities of phenolic monoterpenes, have shown activity against viruses, bacteria, food-derived microbial strains and fungi. Pervious works (Ghasemi Pirbalouti et al., 2009a) showed that essential oils of *T. daenensis* and *Thymbra spicata* (Elam ecotype) flowers exhibited antibacterial activities against *L. monocytogenes* from chicken meat. In a previous study, the minimum inhibitory concentration (MIC > 50 % growth inhabitation) against *L. monocytogenes* for *T. daenensis* and *T. spicata* were 0.700 and 1.7 mg/ml, respectively. Fazeli et al. (2007) studied antimicrobial effects of two medicinal plants (*R. coriaria* L. and *Z. multiflora* Boiss.) used in Iranian traditional medicine were

Table 2. Antibacterial tests of the investigated plants in agar diffusion assay (100 µg/disc).

Plant species	Extraction	*E.c*	*S.a*	*P.a*	*K.p*	*E.c O157:H7*	*B.c*	*L.m*	*Y.e*	*C.c*	*C.j*	*C.a*
Achillea kellalensis Boiss. and Hausskn.	Ethanol extract	21	11	10	9				13			
	Essential oil	18	17	12	13							
Arnebia euchroma (Royle.) Johnston.	Ethanol extract					7	13	14				13
	Aqueous extract						10	12	16			12
Dracocephalum multicaule Montbr and Auch.	Ethanol extract	22			12				11	23	17	
Echiophora platyloba L.	Ethanol extract	12	9	10	16				12	12		
Heracleum lasiopetalum Boiss	Ethanol extract	18	9	11	13				16			
	Essential oil	17	12	15	14					9	23	
Hypericum scabrum L.	Ethanol extract					30	14	12				10
	Aqueous extract						18	11	9			11
Kelussia odoratissima Mozaff.	Ethanol extract	10		12	10	9	20	13	10			13
	Essential oil	16	9	16	17					9	14	
Mentha longifolia (L.) Hudson.	Ethanol extract	14	10	12	9				9			
	Essential oil	17	14	16	12							
Myrtus communis L.	Ethanol extract						19	12				10
	Essential oil					12	28	15				15
Pistachia atlanta Desf.	Ethanol extract					22	27	12				12
Quercus branti Lindley.	Ethanol extract											
Salvia hydrangea DC.	Ethanol extract						16	13				11
	Essential oil					14	30	13				17
Satureja bachtiarica Bung.	Ethanol extract	22	14	13	12							
	Essential oil	23	21	22	14		17	14			15	16
Thymus daenensis Celak.	Ethanol extract	16	8	16	14	12	14	13				11
	Essential oil	18	22	17	19	7	25	16	15			19
Stachys lavandulifolia Vahl.	Ethanol extract	12	13		14							
Ziziphora tenuior L.	Ethanol extract	18	11		8				10			

E.c: *Escherichia coli* ; P.a: *Pseudomonas aeruginosa*; S.a: *Staphylococcus aureu*; K.b: *Klebsiella pneumoniae;* E.c O157:H7*: Escherichia coli* O157:H7; Y.e: *Yersinia enterocolitica*; B.c: *Bacillus cereus*; L.m: *Listeria monocytogenes*; C.c: *Campylobacter coli*; C.j: *Campylobacter jejuni*; C.a: *Candida albicans*.

investigated against some pathogenic food-borne bacteria. The minimum inhibitory concentrations of *R. coriaria* and *Z. multiflora* were determined against several strains of Gram-positive and Gram-negative bacteria. They have reported that *B. cereus* was found to be the most sensitive bacteria to *R. coriaria* showing the MIC of 0.05%., while *S. aureus* and *Proteus vulgaris* ranked next with 0.10% followed by *Shigella flexneri*, *E. coli* and *Salmonella typhi* with MIC of 0.20%.

According to a report (Rasooli et al., 2006) extract and essential oils of *Thymus erioealyx* and *Thymus porlock* inhibited the growth of *L. monocytogenes*. The essential oil and extract of some aromatic plants (for example mint family, *Lamiaceae*) with a higher percentage of cavracrol and thymol have a higher efficacy against strain bacterial (Rasooli et al., 2006).

The result of a study (Ghasemi Pirbalouti et al., 2009b) showed that extract and essential oil of *S. bachtiarica*, *Scrophularia deserti* and *Zizyphus spina-christi* inhibited the growth of *C. albicans*. Ghasemi Pirbalouti et al. (2009a) reported that the essential oils of *T. daenensis* and *T. spicata* ($MIC_{\geq 50\ \%}$ = 0.63 µl ml^{-1} and $MLC_{\geq 99.9\ \%}$ = 22 µl ml^{-1}) and ethanol extract of *Mentha longifolia* showed higher of inhibition against the *Saprolegnia parasitica* than the other extracts. Kokoska et al. (2002) reported that the ethanol extract of *Salvia officinalis* had strong antimicrobial activity against *B. cereus*, *E. coli*, and *S. aureus*.

According to a report (Rasooli et al., 2006) extract and essential oils of *T. erioealyx* and *T. porlock* inhibited the growth of *L. monocytogenes*. The essential oil and extract of some aromatic plants (for example mint family, Lamiaceae) with a higher percentage of cavracrol and thymol have a higher efficacy against strain bacterial (Rasooli et al., 2006).

Conclusions

It can be said that the extract and essential oil of some of the medicinal plants from high altitude of Bakhtiari Zagross Mountains could be used as natural antibacterial agents in the food preservation and human health.

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