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ANTIMICROBIAL ACTIVITY OF SOME IRANIAN MEDICINAL PLANTS

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Abstract - The major aim of this study was to determine the antimicrobial activity of the extracts of eight plant species which are endemic in Iran. The antimicrobial activities of the extracts of eight Iranian traditional plants, including *Hypericum scabrum, Myrtus communis, Pistachia atlantica, Arnebia euchroma, Salvia hydrangea, Satureja bachtiarica, Thymus daenensis* and *Kelussia odoratissima*, were investigated against *Escherichia coli O157:H7, Bacillus cereus, Listeria monocytogenes* and *Candida albicans* by agar disc diffusion and serial dilution assays. Most of the extracts showed a relatively high antimicrobial activity against all the tested bacteria and fungi. Of the plants studied, the most active extracts were those obtained from the essential oils of *M. communis* and *T. daenensis.* The MIC values for active extract and essential oil ranged between 0.039 and 10 mg/ml. It can be said that the extract and essential oil of some medicinal plants could be used as natural antimicrobial agents in food preservation.

Keywords: Myrtus communis, Thymus daenensis, Iranian traditional herbs, food, antibacterial and antifungal activity

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INTRODUCTION

Food spoilage is one of the most important issues facing the food industry. In fact, food-borne illnesses are a global problem, even in developing and developed countries. Food spoilage or deterioration is predominantly caused by the growth of microorganisms. Many pathogenic microorganisms, including *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Campylobacter jejuni*, *Candida* spp., *Zygosaccharomyces* spp., *Fusarium* spp., *Aspergillus* spp., *Rhizopus* spp., *Penicillium* spp., and *Salmonella* spp. have been identified as the causal agents of food-borne diseases or food spoilage (Betts et al., 1999; Solomakos et al., 2008).

Listeria monocytogenes is a Gram-positive bacterium responsible for the severe food-borne illness, listeriosis. This disease is primarily transmitted through various foods, fish, dairy products, cured or processed meat, egg, poultry, seafood, salad, fruits and vegetables (Garcia et al., 2004). Among severe infections, listeriosis has been associated with a mortality rate as high as 30–40% (Datta, 2003).

Bacillus cereus is a species of Gram-positive bacteria inhabiting numerous environments including soil, plant materials and many foods. Due to their spore-forming ability they can pose problems in the food industry (Andersson et al., 1995). In addition, the production of toxins in foods (emetic) or in the human intestine can lead to emesis or diarrhea, respectively. At least three enterotoxins are involved in the pathogenesis of *B. cereus* diarrheal gastroenteritis (Granum and Lund, 1997).

E. coli O157:H7 is recognized as an important cause of food-borne disease. This pathogen is a Gram-negative, facultative anaerobe bacterium,

with a low infection dose of 50–5 organisms (Betts, 2000). According to reports, less than 1% of these food-borne diseases were due to *E. coli*.

Candida albicans, which exists as a commensal organism in the mucocutaneous cavities of the skin, vagina and intestine in humans, can cause infections under altered physiological and pathological conditions, such as infancy, pregnancy, diabetes, prolonged broad-spectrum antibiotic treatment, steroidal chemotherapy and AIDS (Friedman et al., 2000; Kennedy et al., 2000).

Antibiotic resistance has become a global concern. In recent years there is increasing incidence of multiple resistance in human pathogenic microorganisms, largely due to the indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases (Westh et al., 2004). This has forced scientist to search for new antimicrobial substances from various sources like medicinal plants. Search for new antibacterial agents should be continued by the screening of many plant families (Parekh and Chanda, 2007).

The antifungal and antibacterial activity exhibited by the extracts and essential oils 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) against food-borne pathogens. *Hypericum scabrum* L., *Myrtus communis* L., *Pistachia atlantica* Desf., *Arnebia euchroma* (Royle.) Johnston., *Salvia hydrangea* DC., *Satureja bachtiarica* Bunge., *Thymus daenensis* Celak., and *Kelussia odoratissima* Mozff. have been utilized as traditional medicines by the indigenous people of Chaharmahal va Bakhtiari, Southwest Iran (Ghasemi Pirbalouti, 2009). This study aimed to determine the antimicrobial activity of the extracts of eight plant species which are endemic Iranian plants.

MATERIAL AND METHODS

Plant material

An ethnobotanical survey was conducted in Chaharmahal va Bakhtiari Province, South-West of Iran. 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 plants were collected from the mountain areas of Zagross, Chaharmahal va Bakhtiari district, during May – September, 2008. Their identity was confirmed and voucher specimens were deposited at the Research Centre of Medicinal and Aromatic Plants, Islamic Azad University, Shahrekord Branch, Iran.

Extract preparation

Dried plant materials were powdered (200 g) and subjected to hydro-distillation (in 2 000 ml distilled water) for 4 h, using a Clevenger-type apparatus according to the method recommended in British pharmacopoeia (British Pharmacopoeia, 1988). The leaves, roots and flowers of some of the plants, shade dried and ground into a powder (100 g), macerated in 200 ml of 80% ethanol, filtered and dried at 35°C under a rotary vacuum (Model Zirbus 302®, Italy). The extract samples were stored in universal bottles and refrigerated at 4°C prior to use.

Bacterial and fungal strains

The two Gram-positive (*L. monocytogenes* and *B. cereus*) and one Gram-negative (*E. coli* O157:H7) bacterial strains and one fungal strain (*C. albicans*) were all clinical isolates obtained from the Food Microbiology Laboratory, Veterinary Medicine Faculty, Islamic Azad University of Shahrekord Branch, Iran. They were identified using conventional morphological as well as biochemical tests. Stock cultures of the bacteria were kept in 20% glycerol PBS (phosphate buffered saline) at -70°C. Active cultures were generated by inoculating

100 µl of the thawed microbial stock suspensions into 5 ml of nutrient broth (Merck, Germany), followed by overnight incubation at 37°C. The yeast was cultured overnight at 35°C in Sabouraud dextrose agar (SDB) (Merck, Germany). Each microorganism was suspended in sterile saline and diluted at ca. 10^7 colony-forming unit (CFU/ml).

Antimicrobial test

Disc diffusion assay

The disc diffusion method of Iennette (1985) was used with some modification to determine the rate of growth inhibition of bacteria and fungi by the examined plant extracts and essential oils. BHI agar and Sabouraud dextrose agar (Merck, Germany) were used to prepare the culture medium and autoclaved at 121°C for 15 min. Plates (8-cm diameter) were prepared with 10 ml agar inoculated with 1 ml of each microbial suspension. The extracts were dissolved in dimethyl sulfoxide (DMSO, 20 µl) before testing for antimicrobial activity. Sterile paper discs (6 mm in diameter) were impregnated with 60 µl of dilutions of known extract concentrations (100 µg/disc) and incubated at 37°C for 48 h. The plates contained *L. monocytogenes* incubated at 4° C for 48 h. A disc (6 mm diameter) of erythromycin (10 µg) was used as a positive control. Amphotericin B (5 mg/ml) was dissolved in DMSO and served as a positive control. Microbial growth inhibition was determined as the diameter of the inhibition zones around the discs (mm). The growth inhibition diameter was the average of three measurements, taken at three different directions. All tests were performed in triplicate.

Serial dilution

The minimal inhibitory concentration (MIC) value was determined using serial dilution assay. The MIC was defined as the lowest concentration of the compound to inhibit the growth of 50% of microorganisms. All extracts were initially tested at 10 000 µg/ml and serially diluted to 39 µg/ml. Each tube was inoculated with 5 ml of microbial suspension at a density of 10^7 CFU/ml and incubated at 37°C for 48 h. The plates contained *L. monocytogenes* incubated at 4°C for 48 h. The growth of microorganisms was observed as turbidity determined by the measure of optical density at 600 nm (Eppendorf spectrophotometer, AG, Germany). Erythromycin and amphotericin B were included as positive controls in each assay. Extract-free solution was used as a negative control. Control tubes were incubated under the same condition. All assays were carried out in triplicate. The inhibition demonstrated by the extracts is expressed by the following equation (Zampini et al., 2005):

$$\text{Inhibition \%} = [(OD_c - OD_t) / OD_c] \times 100$$

where OD_c is the OD_{600} for the negative control (containing no extract) and OD_t is the OD_{600} for the sample treated with the antimicrobial compounds.

RESULTS

Ethnobotanical survey

The results of the survey are presented in Table 1.

Antibacterial test

The growth inhibition value of extracts and essential oils on microbial strains are shown in table 2. The extracts from the different plant species studied showed antimicrobial activities, with the diameters of the inhibition zone ranging from 7 to 30 mm. There were significant differences ($P \leq 0.05$) in the antimicrobial activities of the plant extracts. Among the plants tested, the essential oils of *M. communis* and *T. daenensis* showed the best antimicrobial activity. These were followed by the ethanol extracts of *H. scabrum* and *P. atlantica,* the essential oil of *S. hydrangea* and erythromycin and amphotericin B which exhibited strong inhibitory activities (Table 2). The results showed that most of the extracts and essential oils could effectively inhibit the growth of *B. cereus*.

Among the plants that were tested, only erythromycin and the ethanol extract of *P. atlantica* and *H. scabrum* could effectively inhibit the growth

Table 1. Ethnobotany of medicinal plants used in Chaharmahal & Bakhtiari districts, Iran. *Habitat: T: Tree, H: Herb

Scientific name	Family name	Local name	Habitat*	Parts used	Uses/ailments treated
Arnebia euchroma (Royle.) Johnston.	Boraginaceae	Sorya	H	rhizome, root	Burn wound, anti-eczema, antimicrobial, anti- inflammatory
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
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
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

of *E. coli* O157:H7; erythromycin and the essential oils of *M. communis* and *T. daenensis* could effectively inhibit the growth of *L. monocytogenes*. Interestingly, the essential oils of *T. daenensis*, *M. communis*, *S. hydrangea* and *S. bachtiarica* showed promising inhibitory activity against *C. albicans* (Table 2).

Subsequent experiments were conducted to determine the minimal inhibitory concentration of all selected plant extracts and essential oils. The results are presented in Tables 3 and 4. Among the plants tested, *T. daenensis*, *S. hydrangea* and *S. bachtiarica* showed the best antimicrobial activities (Table 3). Also, the essential oils of *M. communis* and *K. odoratissima* and the ethanol extract of *T. daenensis*, *M. communis*, *S. hydrangea* and *H. scabrum* showed promising antimicrobial activities against *L. monocytogenes*, *B. cereus* and *C. albicans* (Table 3). The MIC (>50% growth inhibition) for the extracts and essential oils of the medicinal plants is presented in Table 4. The MIC values for active extracts and essential oils ranged between 0.039 and 10 mg/ml. The results obtained appeared to confirm the antimicrobial potential of the plants investigated. The essential oil of *T. daenensis* showed the best MIC value and activity against three bacteria strains and yeast used.

DISCUSSION AND CONCLUSION

The presented results show that Gram-positive bacteria (*L. monocytogenes* and *B. cereus*) were more sensitive than Gram-negative bacteria (*E. coli* O157:H7) (Table 2-4). The antimicrobial activities of the extracts and essential oils of the plants varied in relation to the test organisms. The most active was a 10 mg/ml concentration that inhibited completely the growth of all Gram-positive bacteria and yeast. Cos et al., 2006, reported that Gram-negative bacteria are generally more resistant compared to the Gram-positive ones. Also, Shan et al., 2007 reported that Gram-positive bacteria (*L. monocytogenes*, *Staphylococcus aureus* and *B.*

Table 2. Result of antimicrobial tests of the investigated plants in agar diffusion assay (100 µg/disc). [a:] Diameter of inhibition zone in mm; [b:] part used (organ tested): FL: flower; LE: leaves; ST: stem; GM: Gum; RT: Root; E.c: *Escherichia coli O157:H7*; B.c: *Bacillus cereus*; L.m: *Listeria monocytogenes*; C.a: *Candida albicans*; -: no inhibition

Plant species	Extraction	[a]*E.c*	[a]*B.c*	[a]*L.m*	[a]*C.a*	b
Myrtus communis L.	Ethanol extract	-	19	12	10	LE
	Essential oil	12	28	15	15	
Thymus daenensis Celak	Ethanol extract	12	14	13	11	FL, LE
	Essential oil	7	25	16	19	
Pistacia atlantica Desf.	Ethanol extract	22	27	12	12	GM
Satureja bachtiarica Bunge.	Essential oil	-	17	14	16	LE
Arnebia euchroma (Royle.) Johnst.	Ethanol extract	7	13	14	13	RT
	Aqueous extract	-	10	12	12	
Hypericum scabrum L.	Ethanol extract	30	14	12	10	FL
	Aqueous extract	-	18	11	11	
Salvia hydrangea DC.	Ethanol extract	-	16	13	11	FL
	Essential oil	14	30	13	17	
Kelussia odoratissima Mozff	Aqueous extract	9	20	13	13	LE, ST
Erythromycin (10 µg)	-	17	18	16	-	-
Amphotericin B (5 mg/ml)	-	-	-	-	16	-

cereus) were generally more sensitive to the tested extracts than Gram-negative (*Escherichia coli* and *Salmonella anatum*). *S. aureus* was the most sensitive, while *E. coli* was the most resistant. In the serial dilution assay *C. albicans* was more sensitive than the bacterial strains.

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. It 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 possess this type of 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).

The strongest activity (MIC 0.039 mg/ml) was shown by the essential oils of *T. daenensis* and *S. bachtiarica* against *B. cereus* and *C. albicans*, and

Table 3. Effect of extracts and essential oils on growth microbial strains by serial dilution assay (10 mg/ml). E.c: *Escherichia coli* O157:H7; B.c: *Bacillus cereus*; L.m: *Listeria monocytogenes*; C.a: *Candida albicans*.

Plant species	Extraction	[a]*E.c*	[a]*B.c*	[a]*L.m*	[a]***C.a***
Myrtus communis L.	Ethanol extract	32.37	68.82	65.64	**83.67**
	Essential oil	48.16	77.59	72.82	**91.16**
Thymus daenensis Celak	Ethanol extract	39.56	87.07	68.52	**88.27**
	Essential oil	72.43	92.20	77.30	**96.33**
Pistacia atlantica Desf.	Ethanol extract	39.22	59.27	45.77	**75.03**
Saturja bachtiarica Bunge.	Essential oil	68.25	74.09	71.72	**89.71**
Arnebia euchroma (Royle.) Johnst.	Ethanol extract	37.69	57.67	73.91	**80.52**
	Aqueous extract	37.97	53.62	71.09	**64.13**
Hypericum scabrum L.	Ethanol extract	37.13	56.21	62.88	**80.67**
	Aqueous extract	35.82	61.57	52.81	**58.41**
Salvia hydrangea DC.	Ethanol extract	38.47	66.22	70.13	**85.79**
	Essential oil	54.84	67.44	71.41	**95.25**
Kelussia odoratissima Mozff	Aqueous extract	42.61	62.89	74.55	**84.19**

the essential oils of *M. communis*, *S. hydrangea* against *L. monocytogenes* and *C. albicans*. In this study, the extracts and essential oils were significantly more active against Gram-positive bacteria and yeast (MICs ranging from 0.039 to 10 mg/ml) than against Gram-negative bacteria (MICs > 2.5 mg/ml). Good activity was also observed in the essential oils of *T. daenensis* and *S. bachtiarica*, which inhibited microbial growth from 0.039 to 2.5 mg/ml. Other extracts showed only slight inhibition of the tested microorganisms.

Some studies claim that the phenolic compounds present in spices and herbs might also play a major role in their antimicrobial effects (Hara-Kudo et al., 2004). There has been no large scale, systematic investigation of the relationship between bacterial inhibition and the 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.

Table 4. MIC (50% growth inhabitation) for extracts and essential oils of medicinal plants (mg/ml). E.c: *Escherichia coli* O157:H7; B.c: *Bacillus cereus*; L.m: *Listeria monocytogenes*; C.a: *Candida albicans*.

Plant species	Extraction	[a]*E.c*	[a]*B.c*	[a]*L.m*	[a]*C.a*
		MIC	MIC	MIC	MIC
Myrtus communis L.	Ethanol extract	>10	0.625	2.5	0.625
	Essential oil	10	0.625	<0.039	<0.039
Thymus daenensis Celak	Ethanol extract	>10	0.625	2.5	<0.039
	Essential oil	2.5	0.039	0.156	<0.039
Pistacia atlantica Desf.	Ethanol extract	>10	0.625	>10	0.625
Saturja bachtiarica Bunge.	Essential oil	2.5	<0.039	0.625	0.039
Arnebia euchroma (Royle.) Johnst.	Ethanol extract	>10	2.5	0.039	0.625
	Aqueous extract	>10	10	0.156	2.5
Hypericum scabrum L.	Ethanol extract	>10	2.5	625	0.039
	Aqueous extract	>10	2.5	10	2.5
Salvia hydrangea DC.	Ethanol extract	>10	0.156	2.5	<0.039
	Essential oil	10	0.625	<0.039	<0.039
Kelussia odoretascima Mozff	Aqueous extract	>10	0.156	0.625	0.039

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 *Thymu*s 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., 2009) showed that the essential oils of *T. daenensis* and *Thymus* spp. (Elam ecotype) flowers exhibited antibacterial activities against *L. monocytogenes* from chicken meat. In a previous study, the minimum inhibitory concentration (MIC > 50 % growth inhibition) against *L. monocytogenes* for *T. daenensis* and *Thymus* spp. were 0.700 and 1.7 mg/ml, respectively.

Fazeli et al., 2007 studied the antimicrobial effects of two medicinal plants (*Rhus coriaria* L. and *Zataria multiflora* Boiss.) used in Iranian traditional medicine which were investigated against some pathogenic food-borne bacteria. The minimum inhibitory concentrations of *Rhus coriaria* and *Zataria multiflora* were determined against several strains of Gram-positive and Gram-negative bacteria. They have reported that *Bacillus cereus* was found to be the most sensitive bacteria to *Rhus coriaria,* showing a 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 a MIC of 0.20%.

According to a report (Rasooli et al., 2006) the extracts and essential oils of *Thymus erioealyx* and *Thymus porlock* inhibited the growth of *L. monocytogenes*. The essential oils and extracts of some aromatic plants (e.g. the 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., 2008) showed that the extracts and essential oils of *S. bachtiarica*, *Scrophularia deserti* and *Zizyphus spina-christi* inhibited the growth of *C. albicans*. Ghasemi Pirbalouti et al., 2009 reported that the essential oils of *T. daenensis* and *Thymus khuzestanicum* (MIC $_{\geq 50\%}$ = 0.63 µl ml^{-1} and MLC $_{\geq 99.9\%}$ = 22 µl ml^{-1}) and the ethanol extract of *Mentha longifolia* showed higher of inhibition against *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 the findings of this study, most of the extracts showed relatively high antimicrobial activity against all the tested bacteria and fungi. The essential oils of *S. bachtiarica* and *T. daenensis* leaves and flowers had antimicrobial activities. The present study suggests that the essential oil of these plants is a potential source of natural antibacterial agents. After this screening experiment, further work should be performed to describe the antimicrobial activities in more detail as well as their activity *in vivo*. Also phytochemical studies will be necessary to isolate the active constituents and evaluate the antibacterial activities against a wide range of bacteria population.

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