Journal of Medicinal Plants Research Vol. 5(14), pp. 3067-3073, 18 July, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

Sensitivity of *Lactococcus garvieae* isolated from rainbow trout to some Iranian medicinal herbs

M. A. Goudarzi[1], B. Hamedi[1], F. Malekpoor[1], R. Abdizadeh[2], A. Ghasemi Pirbalouti[2]* and M. Raissy[3]

[1]Shahrekord Branch, Islamic Azad University, Club of Young Researchers, Shahrekord, Iran.
[2]Shahrekord Branch, Islamic Azad University, Department of Medicinal Plants, Research Centre of Medicinal Plants and Ethnoveterinary, Shahrekord, P. O. Box: 166, Iran.
[3]Shahrekord Branch, Islamic Azad University, Department of Fish Health, Faculty of Veterinary Medicine, Shahrekord, Iran.

Accepted 23 March, 2011

***Lactococcus garvieae* is one of the most important bacterial pathogens that affect different farmed fish species in many countries. Some of the medicinal plants and their extracts have an antibacterial activity. In this study, antibacterial activity of ethanol extracts and essential oils of fifteen Iranian medicinal plants including, *Satureja bachtiarica* Bunge., *Thymus daenensis* Celak., *Stachys lavandulifolia* Vahl., *Zataria multifora* Boiss., *Thymbra spicata* L., *Teucrium polium* L. (Lamiaceae); *Myrtus communis* L. (Myrtaceae); *Heracleum lasiopetalum* Boiss., *Kelussia* *odoratissima* Mozaff., *Bunium persicum* (Boiss.) K.-Pol., *Echiophora platyloba* L. (Apiaceae); *Punica granatum* L. (Punicaceae); *Qurcus branti* Lindle (Fagaceae); *Glycyrrhiza glabra* L., *Alhagi maurorum* L. (Fabaceae) were investigated against *Lactococcus garvieae* isolated from rainbow trout (*Oncorhynchus mykiss*) by agar disc diffusion and serial dilution assays. Just the extracts of *M. communis* and *T. daenensis* showed relatively antibacterial activity against *L. garvieae*. Almost the essential oils showed strong antibacterial activity against fish pathogen. The results obtained appeared to confirm the antibacterial potential of the essential oils of *B. persicum*, *Z. multifora*, *T. daenensis, S. bachtiarica* and *T. spicata* against *L. garvieae* isolated from rainbow trout. The MICs and MLCs of *Z. multifora* essential oil against *L. garvieae* were found as 4-8 µl/ml.**

Key words: *Lactococcus garvieae*, medicinal plants, ethanol extract, antibacterial activity, essential oil.

INTRODUCTION

The genus *Lactococcus* belongs to the family Streptococcaceae and is characterized by facultatively anaerobic Gram-positive bacteria, arranged in pairs and short chains, with the ability to produce lactic acid (Furutan et al., 1991; Fihman et al., 2005). It is described in 1985 after the division of the *Streptococcus* genus, which included a group of agents known as lactic streptococci represented by agents isolated in dairy cattle and milk products (Schleifer et al., 1985). *Lactococcus garvieae*, 1 of 8 species in the genus, the most important species of the *Lactococcus* genus, is non-motile, ovoid cocci, produces a-haemolytic colonies on blood agar, and is oxidase and catalase negative, non-acid fast, and non-sporulating (Ravelo et al., 2001; Vendrell et al., 2006). Lactococcosis caused by *L. garvieae* is an important infectious disease of farmed rainbow trout (*Oncorhynchus mykiss*) in many countries when water temperature rises above 16°C in summer months. In addition, this pathogen is one of the most important bacterial pathogens that affect different farmed fish species in many countries, although its major impact is on the trout farm industry (Elliot et al., 1991; Facklam and Elliot, 1995). In addition to farmed fish, this microorganism has also been isolated from a wide range of wild fish species, from both fresh and marine water, as well as from giant fresh water prawns (Fefer et al., 1998)

*Corresponding author. E-mail: ghasemi@iaushk.ac.ir or ghasemi955@yahoo.com. Tel: 00983813361060. Fax: 00983813361031.

and from wild marine mammals (Li et al., 2008). The host range of *L. garvieae* is not limited to aquatic species. This agent has also been identified in cows and water buffalos with subclinical mastitis (Wang et al., 2007; Van Hijum et al., 2008) and from cat and dog tonsils (Hakenbeck et al., 2001). In humans, it has been isolated from the urinary tract, blood, and skin and from patients with pneumonia, endocarditic or septicemia (Dong et al., 2001; Fukiya et al., 2004; Zhang et al., 2008; Michel et al., 2007). Recently, intestinal disorders in humans have been associated with the consumption of raw fish contaminated with this pathogen (Coenye et al., 2003), which suggests that *L. garvieae* could be considered as a potentially zoonotic bacterium (Fefer et al., 1998; Coenye et al., 2003).

The rapidly expanding aquaculture industry in Iran has suffered heavy economic losses due to bacterial pathogens, particularly *Streptococcus iniae* and *L. garvieae*, which are the major agents of Lactococcosis in rainbow trout (Akhlaghi and Keshavarzi, 2002; Akhlaghi and Mahjoor, 2004). The losses produced can exceed approximately 50-80% of the total production (Ghittino and Prearo, 1992). The antibiotics most often used to control Lactococcosis in rainbow trout outbreaks have been erythromycin, oxytetracycline, amoxicillin and low-level doxycycline (Munday, 1994). The reference strains of *L. garvieae* are sensitive to erythromycin, with a minimum inhibitory concentration (MIC) of 0.12 mg/ml (Elliot and Facklam, 1996). Recently, outbreaks in Turkey demonstrated that the strains of *L. garvieae* isolated were sensitive to erythromycin, oxacin, ampicillin and chloramphenicol, but were resistant to penicillin and clyndamycin (Diler et al., 2002).The increased public awareness of the negative effects caused by overexposure to synthetic chemicals led to the search for "green solutions", such as organic and synthetic chemical-free food products (Abutbul et al., 2004). To enable organic fish production, it is essential to develop antibacterial treatments based on materials from natural sources. The medicinal herbs contain physiologically active principles that over the years exploited in traditional medicine for the treatment of various ailments as they have anti-microbial properties (Kelmanson et al., 2000; Srinivasan et al., 2001). Antimicrobial properties of herbs have been documented in ancient literature and the interest continues to the present. However, few of these are investigated for their antimicrobial. Several of these spices and their essential oils reported to posses antimicrobial activities including garlic, savory, basil, laurel, mint, cumin, onion, sumac and thyme (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; Shelef, 1983).

Numerous Iranian folklore herbs for example: *Satureja bachtiarica, Thymus daenensis, Myrtus communis, Echinophora platyloba, Zataria multifora*, *Thymbria spicata*, *Bunium persicum*, *Alhagi maurorum*, and *Teucrium polium* have been used for antimicrobial and antiseptic as Iranian traditional medicines or *Unani* medicine (Ghasemi, 2009; Zargari, 1989-1992). Hence, it is necessary to establish the scientific basis for the therapeutic actions of traditional plant medicines as these may serve as the source for the development of more effective drugs. The research reports on these folklore herbs were limited and the antibacterial effects of fifteen herbs have not been studied until now. In this study, it is aimed to determine antibacterial activity of ethanol extracts and essential oils of fifteen Iranian medicinal plants investigated against *L. garvieae* isolated from rainbow trout (*O. mykiss*) by agar disc diffusion and serial dilution assays.

MATERIALS AND METHODS

Plant material

Nine Iranian medicinal herbs which comprise *S. bachtiarica* Bunge., *T. daenensis* Celak., *Stachys lavandulifolia* Vahl., *M. communis* L., *Heracleum lasiopetalum* Boiss., *Kelussia odoratissima* Mozaff., *E. platyloba* L., *Qurcus branti* Lindle and *Glycyrrhiza glabra* L. were collected from mountain areas of Zagross, Chaharmahal va Bakhtiari District (altitude: 2000-2500 m asl; latitude: 30°-31°; longitude: 50°-51°), during May–Sep 2009. Their identity was confirmed using the monographs by Ghahraman (1987–1989), Mozaffarian (2006) and Rechinger (1963-1998), and voucher specimens were deposited at the Researches Centre of Medicinal Plants, Islamic Azad University, Shahrekord Branch, Iran.

Six Iranian medicinal herbs including *Z. multifora* Boiss., *T. polium* L., *B. persicum* (Boiss.) K.-Pol., *P. granatum* L., *A. maurorum* L. and *T. spicata* L. were collected from a well-known market for Iranian herbal medicines in Shahrekord, Isfahan, Kerman and Ilam, Iran. The scientific names and tested parts of the fifteen plant materials are in Table 1.

Preparation of extracts

Dried plant materials were grinded into powder (200 g) and subjected to hydro-distillation (2000 ml distillated water) for 3 h using a Clevenger- type apparatus according to the method recommended in British Pharmacopoeia (British Pharmacopoeia, 1988). Leaves, seed and flowers of some of the plants shade dried and, grinded into powder (100 g), macerated in 200 ml of ethanol 96% and filtered were dried at 35°C under rotary vacuum (Model Zirbus 302®, Italy). The extract samples were stored in universal bottles and refrigerated at 4°C prior to use.

Bacterial strain

L. garvieae was isolated from infected rainbow trout (*O. mykiss*) from a commercial aquaculture farm in Iran. It was kindly given by Dr. Namatollahi, Department of Fish Health, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran. The isolate was identified as *L. garvieae* using conventional morphological as well as biochemical tests. Bacteria were kept frozen in 15% glycerol and 85% saline solution or Brain Heart Infusion (BHI, Merk, Germany) broth, in aliquots, at -70°C until used. For infection trials, 100 ml of BHI broth was inoculated with 50 µL of the frozen isolate. The cultures were shaken (100 rpm) at 27°C for 24 h. Absorbance at 600 nm of known bacterial densities was determined to obtain a standard calibration curve (data not shown). An initial bacterial suspension containing 10^7 CFU/ml was made from the flask broth culture. Subsequent dilutions were made from the above suspension, and then used in tests

Table 1. Designation of studied plant extracts, their family and botanical names and used parts.

Scientific name	Family name	Local name	Extract	Components	Parts used
Punica granatum L.	Punicaceae	Golnar	Ethanol	Polyphenols: Pomegranatate	Flowers
Qurcus branti Lindley.	Fagaceae	Balout	Ethanol	Tannins	Seed (flour)
Echinophora platyloba DC.	Apiaceae	Khosharizeh	Essential oil	Monoterpenes: trans-β-ocimene	Aerial parts
Heracleum lasiopetalum Boiss.	Apiaceae	Keresom	Ethanol	Sesquiterpene hydrocarbons: Germacrene-D	Fruits
Kelussia odoratissima Mozaff.	Apiaceae	Kelus	Ethanol	Z-ligustilide	Leaves
Glycyrrhiza glabra L.	Fabaceae	Shirin bayan	Ethanol	Glycyrrhizinic acid	Roots
Zataria multifora Boiss.	Lamiaceae	Avishan	Essential oil	Phenols: thymol, carvacrol, etc	Aerial parts (Inflorescence)
Thymbra spicata L.	Lamiaceae	Avishan	Essential oil	Phenols: thymol, carvacrol, etc	Aerial parts (Inflorescence)
Bunium persicum (Boiss.) K.-Pol.	Apiaceae	zireh	Essential oil	γ-terpinen-7-al, cuminaldehyde, γ-terpinene	Fruits
Teucrium polium L.	Lamiaceae	chez	Essential oil	α-pinene, linalool	Aerial parts
Alhagi maurorum L.	Papilionaceae	Khar shotor	Essential oil	Alhagidin, alhagitin, quercetin, catechin	Aerial parts
Satureja bachtiarica Bung.	Lamiaceae	Marzeh Koohi	Ethanol Essential oil	Phenols: thymol, carvacrol	Aerial parts
Stachys lavandulifolia Vahl.	Lamiaceae	Lolopashmak	Ethanol Essential oil	Sabinene, α-pinene, β-myrcene	Flowers
Thymus daenensis Celak.	Lamiaceae	Oushon	Ethanol Essential oil	Phenols: thymol, carvacrol	Aerial parts (Inflorescence)
Myrtus communis L.	Myrtaceae	Mord	Ethanol Essential oil	α-pinene,, 1,8-cineole, myrtenyl acetate	Leaves

Antimicrobial test

Disc diffusion assay

The disc diffusion method of Lennette (1985) was used with some modification to determine rate of inhibition growth of bacteria by plant essential oils and ethanol extracts. BHI agar (Merck, Germany) was used to prepare the culture medium and was autoclaved at 121°C for 15 min. Briefly, plates (8 cm in diameter) were prepared with 10 ml agar inoculated with 1 ml of each bacterial suspension. Sterile paper discs (6 mm in diameter) were impregnated with dilutions of known extract concentrations and incubated at 35°C for 24 h. The ethanol extracts and essential oils were dissolved in dimethyl sulfoxid (DMSO) before the test for antimicrobial activity. Erythromycin concentrations used as positive controls. Bacterial 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

Table 2. Antibacterial activity of used ethanol extracts against *L. garvieae.*

Ethanol extract	Concentrations (µg/ml)				
	1000	500	250	125	62.5
Punica granatum L.	-	-	-	-	-
Qurcus branti Lindley.	-	-	-	-	-
Echinophora platyloba DC.	-	-	-	-	-
Heracleum lasiopetalum Boiss.	-	-	-	-	-
Kelussia odoratissima Mozaff.	-	-	-	-	-
Glycyrrhiza glabra L.	-	-	-	-	-
Satureja bachtiarica Bung.	-	-	-	-	-
Stachys lavandulifolia Vahl.	-	-	-	-	-
Thymus daenensis Celak.	7 [a]	4	2	-	-
Myrtus communis L.	12	8	4	-	-
Erythromycin	20	18	15	15	10
DMSO	-	-	-	-	-

[a:] Diameter of inhibition zone in mm. - : no inhibition. Values are means of three replications.

Table 3. Antibacterial activity of used essential oils against *Lactococcus garvieae.*

Essential oil	Concentrations (µl/ml)				
	20	50	100	200	250
Echinophora platyloba DC.	-	-	-	7 [a]	7
Zataria multifora Boiss.	12	16	18	23	23
Thymbra spicata L.	-	12	14	19	19
Bunium persicum (Boiss.) K.-Pol.	12	15	16	23	25
Teucrium polium L.	-	-	-	-	-
Alhagi maurorum L.	-	-	-	-	-
Satureja bachtiarica Bung.	4	7	8	15	15
Thymus daenensis Celak.	7	14	16	21	23
Myrtus communis L.	-	-	3	10	12
DMSO	-	-	-	-	-

[a:] Diameter of inhibition zone in mm. - : no inhibition. Values are means of three replications.

different directions. All tests were performed in triplicate.

Serial dilution

The minimal inhibitory concentration (MIC) and the minimal lethal concentration (MLC) values were determined by serial dilution assay. The MIC was the lowest concentration at which bacteria failed to grow in liquid media, but viable when 100 µl samples were plated on agar media. The MLC was the lowest concentration at which bacteria failed to grow in liquid media, but were not cultured after 100 µl samples were plated on agar media (Smith-Palmer et al., 1998).

Each tube was inoculated with 5 ml of bacterial suspension at a density of 10^7 CFU/ml and incubated at 37°C for 48 h. The growth of microorganisms was observed as turbidity, determined by the measure optical density at 600 nm, by spectrophotometer (Eppendorf, AG, Germany). Erythromycin was included as positive control 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.

RESULTS

As can be seen in Tables 2 and 3, essential oils and the ethanol extracts obtained from different plant species studied showed antimicrobial activity against *L. garvieae* isolated from rainbow trout (*O. mykiss*) by agar disc diffusion assay. The antibacterial activity of essential oils and ethanol extracts were in the range of less than 8 mm and not higher than 25 mm. There were significant differences ($P \leq 0.05$) in the antibacterial activities of plant essential oils and extracts. Among all essential oils and ethanol extracts, the essential oil of *B. persicum* fruits, *Z. multifora* aerial parts, *T. daenensis* aerial parts and *T. spicata* aerial parts were the most active against *L. garvieae* inhibition. In different concentrations, the growth of *L. garvieae* was inhibited by the essential oils of *B. persicum*, *Z. multifora*, *T. daenensis* and *T. spicata* at strong level, while the other essential oils of ethanol

Table 4. Minimal inhibitory concentration (MIC) and Minimum lateral concentration (MLC) for essential oils and ethanol extracts of fifteen Iranian medicinal herbs against *Lactococcus garvieae.*

Plant	Extract	MIC	MLC
Alhagi maurorum L.	Essential oil	> 1000 µl/ml	> 1000 µl/ml
Bunium persicum (Boiss.) K.-Pol.	Essential oil	8 µl/ml	16 µl/ml
Echinophora platyloba DC.	Ethanol extract	> 1000 µg/ml	> 1000 µg/ml
Echinophora platyloba DC.	Essential oil	> 1000 µl/ml	> 1000 µl/ml
Glycyrrhiza glabra L.	Ethanol extract	> 1000 µg/ml	> 1000 µg/ml
Heracleum lasiopetalum Boiss.	Ethanol extract	> 1000 µg/ml	> 1000 µg/ml
Kelussia odoratissima Mozaff.	Ethanol extract	> 1000 µg/ml	> 1000 µg/ml
Myrtus communis L.	Ethanol extract	> 250 µg/ml	> 500 µg/ml
	Essential oil	> 1000 µl/ml	> 1000 µl/ml
Punica granatum L.	Ethanol extract	> 1000 µg/ml	> 1000 µg/ml
Qurcus branti Lindley.	Ethanol extract	> 1000 µg/ml	> 1000 µg/ml
Satureja bachtiarica Bung.	Ethanol extract	> 1000 µg/ml	> 1000 µg/ml
	Essential oil	8 µl/ml	16 µl/ml
Stachys lavandulifolia Vahl.	Ethanol extract	> 1000 µg/ml	> 1000 µg/ml
Teucrium polium L.	Essential oil	> 1000 µl/ml	> 1000 µl/ml
Thymbra spicata L.	Essential oil	8 µl/ml	16 µl/ml
Thymus daenensis Celak.	Ethanol extract	> 500 µg/ml	> 1000 µg/ml
	Essential oil	8 µl/ml	16 µl/ml
Zataria multifora Boiss.	Essential oil	4 µl/ml	8 µl/ml
Erythromycin	-	8 µg/ml	16 µg/ml

extracts at weak level. Based on these results, the essential oils have a stronger and broader spectrum of antimicrobial activities compared with the ethanol extracts. Positive control (reference antibiotics) generally showed antibacterial activity to our test microorganism. Since final concentrations of all extracts were adjusted with DMSO, it was used as a negative control and there was no inhibition with this control solvent (Tables 2 and 3).

Subsequent experiments were conducted to determine minimal inhibitory concentration (MIC) and minimum lethal concentration (MLC) of all selected plant extracts and essential oils. The results presented in Table 4. The highest level of antibacterial activity demonstrated essential oil of *Z. multifora* against *L. garvieae* (Table 4). The MICs of *Z. multifora* essential oil against *L. garvieae* were found as 4-8 µl/ml. In addition, *B. persicum* (8-16 µl/ml), *T. daenensis* (8-16 µl/ml), *S. bachtiarica* (8-16 µl/ml) and *T. spicata* (8-16 µl/ml) showed the best antibacterial activities against *L. garvieae* (Table 4). Other essential oils and ethanol extracts showed weak inhibition of tested microorganism. *L. garvieae* was resistant to all tested ethanol extracts except *M. communis* exhibiting moderate antibacterial activity with MIC and MLC= 1000 µg/ml. The MIC of erythromycin for *L. garvieae* obtained was 8-16 µg/ml.

DISCUSSION

The highest inhibitory activity was obtained from essential oil of *B. persicum*, *Z. multifora*, *T. daenensis, S. bachtiarica* and *T. spicata* and positive control (reference antibiotics) which inhibited the growth of *L. garvieae*. Similarly, Fazeli et al. (2007) studied antimicrobial effects of two medicinal plants (*Rhus coriaria* L. and *Z. multifora* Boiss.), used in Iranian traditional medicine investigated against some pathogenic food-borne bacteria (*Staphylococcus aureus*, *Proteus vulgaris Shigella flexneri*, *Escherichia coli* and *Salmonella typhi*). Ghasemi et al. (2009) showed that essential oil of *S. bachtiarica* exhibited antifungal activities against *Saprolegnia parasitica* from oils of *S. bachtiarica* and *T. daenensis* exhibited antibacterial activities against *S. aureus*, *E. coli*, *P. aeruginosa* and *Klebsiella pneumonia*. Ghasemi et al. (2010b) reported that essential oils of *M. communis*, *T. daenensis* and *S. bachtiarica* exhibited antimicrobial activities against *E. coli* O157:H7, *Bacillus cereus*, *Listeria monocytogenes* and *Candida albicans*. The results of a study (Turker et al., 2009) showed that the ethanol extract of *Vinca minor* leaves exhibited strong antibacterial activity against *L. garvieae* in comparison with other alcoholic and aqueous extracts from 21 species of herbs from Bolu (Turkey).

In present study, the essential oils of medicinal plants have a stronger and broader spectrum of antimicrobial activities compared with the ethanol extracts. While the opposite results by Turker et al. (2009) were reported. They suggested that use of alcohol as organic solvent provided a higher efficiency in extracting antimicrobial activities compared with water extraction and the use of

alcoholic extracts may be suggested for the natural administration of antibiotics effective in fish disease control (Turker et al., 2009).

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

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 bacteria (Rasooli et al., 2006). Essential oil of *T. daenensis* and *S. bachtiarica* contained high levels of phenolics and exhibited antibacterial activity (Ghasemi, 2009b). 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.

According to the findings of this study, essential oils of *B. persicum*, *Z. multifora*, *T. daenensis, S. bachtiarica* and *T. spicata* had antibacterial activities against *L. garvieae*. In two assays of antibacterial test, the highest level of antibacterial activity was demonstrated by the essential oil of the aerial parts (inflorescence) of *Z. multifora* (MIC = 4 µl/ml; MLC = 8 µl/ml).

In this study it was shown that *L. garvieae* was sensitive to erythromycin with MIC=8 and MLC=16 µg/ml. The antibiotics most often used to control Lactococcosis in rainbow trout outbreaks have been erythromycin, oxytetracycline, amoxicillin and low-level doxycycline (Munday, 1994). The reference strains of *L. garvieae* are sensitive to erythromycin, with a minimum inhibitory concentration (MIC) of 0.12 mg/ml (Elliot and Facklam, 1996). In addition, some sensitivity to ionophores antibiotics described. Recently, outbreaks in Turkey demonstrated that the strains of *L. garvieae* isolated were sensitive to erythromycin, oxacin, ampicillin and chloramphenicol, but were resistant to penicillin and clyndamycin (Diler et al., 2002).

Heavy antibiotic used in aquaculture needs to be reduced and replaced with alternative processes for treating fish diseases to avoid the emergence of antibiotic resistance in pathogenic and environmental bacteria (Sørum and L'Abée-Lund, 2002; Cabello, 2006). Natural substances like thyme oil, clove oil and pine oil were used as alternative bio-herbicides and bio-pesticides in ecological agriculture (Verschwele, 2005; Perez and Lewis, 2006). Similarly, the herbal plants may be used as potential and promising source of pharmaceutical agents against fish pathogens in the organic aquaculture.

The screening results of our study confirm the possible use of Iranian medicinal herbs as a source of antimicrobial agent for this purpose. The present study describes, to our knowledge for the first time, antibacterial activities of fifteen plants against rainbow trout fish pathogen (*L. garvieae*) and the efficacy of some herbs for the treatment of bacterial fish diseases was scientifically verified. In summary, our results indicate that these species of herbs collected, present a significant antimicrobial activity against pathogenic fish bacteria. Finally, the observation that essential oils of *B. persicum* fruits, *Z. multifora* inflorescence, *T. daenensis* inflorescence*, S. bachtiarica* aerial parts and *T. spicata* inflorescence effectively inhibit bacteria provides the aquaculturists with a promising management tool for control or treatment of fish diseases. In addition, further research is needed to determine the active compound of the herbs and the effect of these compounds to the fish metabolism. An alternative approach for a possible practical use of extracts should be also applied. Also further work should be performed to describe the antibacterial activities in more detail, *in vivo*.

REFERENCES

Abutbul A, Golan-Goldhirsh A, Barazani O, Zilberg D (2004). Use of *Rosmarinus officinalis* as a treatment against *Steptococcus iniae* in tilapia (*Oreochromis* sp.). Aquaculture, 238: 97-105.

Akhlaghi M, Keshavarzi M (2002). The occurrence of streptococcosis in the cultured rainbow trout of Fars province. Iranian. J. Vet. Res., 2: 183-189.

Akhlaghi M, Mahjoor MM (2004). Some histopathological aspects of streptococcosis in cultured rainbow trout (*Oncorhynchus mykiss*). Br. Eur. Assoc. Fish Pat., 24: 132-136.

Arora DS, Kaur J (1999). Antimicrobial activity of spices. Int. J. Antimicrob. Agent, 12: 257-262.

British Pharmacopoeia (1988). HMSO. London, 2: 137-138.

Cabello FC (2006). Heavy use of prophylactic antibiotics in aquaculture: A growing problem for human and animal health and for the environment. Environ. Microb., 8: 1137-1144.

Coenye T, Vandamme P (2003). Extracting phylogenetic information from whole-genome sequencing projects: the lactic acid bacteria as a test case. Microbiol., 149: 3507-3517.

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. Tech., 218: 188-193.

Diler O, Altun S, Adiloglu AK, Kubilay A, Isikli B (2002). First occurrence of Streptococcosis affecting farmed rainbow trout (*Oncorhynchus mykiss*) in Turkey. Bull. Eur. Ass Fish Pathol., 22: 21-25.

Dong Y, Glasner JD, Blattner FR, Triplett EW (2001). Genomic interspecies microarray hybridization: rapid discovery of three thousand genes in the maize endophyte, *Klebsiella pneumoniae* 342, by microarray hybridization with *Escherichia coli* K-12 Open Reading Frames. Appl. Environ. Microbiol., 67: 1911-1921.

El-Khateib T, Abd El-Rahman H (1987). Effect of garlic and *Lactobacillus plantarum* on growth of *Salmonella typhimurium* in Egyptian fresh sausage and beef burger. J. Food Protect., 50: 310-311.

Elliot JA, Collins MD, Pigott NE, Facklam RR (1991). Differentiation of *Lactococcus lactis* and *Lactococcus garvieae* from humans by comparison of whole-cell protein patterns. J. Clin. Microbiol., 20: 2731-2734.

Elliot JA, Facklam RR (1996). Antimicrobial susceptibilities of *Lactococcus lactis* and *Lactococcus garvieae* and a proposed method to discriminate between them. J. Clin. Microbiol., 34: 1296-1298.

Facklam RR, Elliot JA (1995). Identification, classification, and clinical relevance of catalase-negative, Gram-positive cocci, excluding the streptococci and enterococci. Clin Microbiol. Rev., 8: 479-495.
Fazeli MR, Amin GHR, Ahmadian Attari MM, Ashtiani H, Jamalifar H, Samadi N (2007). Antimicrobial activities of Iranian sumac and avishan-e shirazi (*Zataria multiflora*) against some food-bornebacteria. Food Control, 18: 646-649.
Fefer JJ, Ratzan KR, Sharp SE, Saiz E (1998). *Lactococcus garvieae* endocarditis: report of a case and review of the literature. Diagn. Microbiol. Infect. Dis., 32: 127-130.
Fihman V, Raskine L, Barrou Z, Kiffel C, Riahi J, Bercot B, Sanson-Le Pors MJ (2005). *Lactococcus garvieae* endocarditis: identification by 16S rRNA and sodA sequence analysis. J. Infect., 52: e3-e6.
Fukiya S, Mizoguchi H, Tobe T, Mori H (2004). Extensive genomic diversity in pathogenic *Escherichia coli* and *Shigella* strains revealed by comparative genomic hybridization microarray. J. Bacteriol., 186: 3911-3921.
Furutan NP, Breiman RF, Fischer MA, Facklam RR (1991). *Lactococcus garvieae* infection in humans: a cause of prosthetic valve endocarditis [C297]. Proceedings of the 91st America Society of Microbiology Conference. Dallas7 Am. Soc. Microbiol., p 109.
Ghahraman A (1987-1989). Flora of Iran. Department of Botany, Institute of and Rangelands Press, Tehran (in Persian).
Ghasemi PA (2009a). Medicinal plants used in Chaharmahal and Bakhtyari districts, Iran. Herba Polonica, 55: 34-38.
Ghasemi PA (2009b). Iranian medicinal and aromatic plants. Islamic Azad University Publishers, Shahrekord, Iran, 1nd edition, p. 550.
Ghasemi PA, Taheri M, Raisee M, Bahrami HR, Abdizadeh R (2009). *In vitro* antifungal activity of plant extracts on *Saprolegnia parasitica* from cutaneous lesions of rainbow trout (*Oncorhynchus mykiss*) eggs. J. Food Agric. Environ., 7: 94-96.
Ghasemi PA, Malekpoor F, Enteshari S, Yousefi M, Momtaz H, Hamedi B (2010a). Antibacterial activity of some folklore medicinal plants used by Bakhtiari tribal in Southwest Iran. Int. J. Biol., 2: 55-63.
Ghasemi PA, Jahanbazi P, Enteshari S, Malekpoor F, Hamedi B (2010b). Antimicrobial activity of some of the Iranian medicinal plants. Arch. Biolog. Sci., 62: 633-642.
Ghittino, C, Prearo M (1992). Report of streptococcosis in rainbow trout (*Oncorhynchus mykiss*) in Italy: preliminary note. Bull. Italian Soc. Fish Pathol., 8: 4-11
Hakenbeck R, Balmelle N, Weber B, Gardes C, Keck W, de Saizieu A (2001). Mosaic genes and mosaic chromosomes: intra- and interspecies genomic variation of *Streptococcus pneumoniae*. Infect. Immun., 69: 2477-2486.
Hara-Kudo Y, Kobayashi A, Sugita-Konishi Y, Kondo K (2004). Antibacterial activity of plants used in cooking for aroma and taste. J. Food Protect., 67: 2820-2824.
Lennette EH (1985). Manual of clinical Microbiology fourth ed. American Association for Microbiology, Washington, DC, pp. 978-987.
Kelmanson JE, Jager AK, Van Staden J (2000). Zulu medicinal plants with antibacterial activity. J. Ethnopharmacol., 69: 241-246.
Li WK, Chen YS, Wann SW, Liu YC, Tsai HT (2008). *Lactococcus garvieae* endocarditis with initial presentation of acute cerebral infarction in a healthy immunocompetent man. Inter. Med., 47: 1143-1146.
Michel C, Pelletier C, Boussaha M, Douet DG, Lautraite A, Tailliez P (2007). Diversity of lactic acid bacteria associated with fish and the fish farm environment, established by amplified rRNA gene restriction analysis. Appl. Environ. Microbiol., 73: 2947-2955.
Mozaffarian V (2006). A dictionary of Iranian plant names. Farhang Mosavar Press, Tehran, Iran (in Persian).
Munday BL (1994). Fish. In: Coopert SB, editor. Antimicrobial Prescribing Guidelines for Veterinarians: a Post Graduate Foundation Publication. University of Sydney, Australia, In association with the National Health and Medical Research Council, pp. 305-325.
Nasar-Abbas SM, Kadir Halkman A (2004). Antimicrobial effect of water extract of sumac (*Rhus coriaria* L.) on the growth of some food borne bacteria including pathogens. Inter. J. Food Microb., 97: 63-69.
Perez EE, Lewis EE (2006). Use of entomopathogenic nematodes and thyme oil to suppress plant-parasitic nematodes on English boxwood. Plant Dis., 90: 471-475.
Ozcan M, Erkmen O (2001). Antimicrobial activity of the essential oils of Turkish plant spices. Eur. Food Res. Tech., 212: 658-660.
Rasooli I, Rezaei MB, Allameh A (2006). Ultrastructural studies on antimicrobial efficacy of thyme essential oils on Listeria monocytogenes. Inter, J, Infect, Dis., 10: 236-241.
Ravelo C, Magarin˜os B, Romalde JL, Toranzo AE (2001). Conventional versus miniaturized systems for the phenotypic characterization of *Lactococcus garvieae* strains. Bull. Eur. Assoc. Fish Pathol., 21: 136-144.
Rechinger KH (1963-1998). Flora Iranica. Austria, Graz: Akademische Druck und Verlagsanstalt, Vol. 1-173.
Schleifer KH, Kraus J, Dvorak C, Kilpper-Balz R, Collins MD (1985). Fischer W. Transfer of *Streptococcus lactis* and related streptococci to the genus *Lactococcus* gen nov. Syst. Appl. Microbiol., 6: 183-195.
Shan B, Cai YZ, Sun M, Corke H (2005). Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. J. Agric. Food Chem., 53: 7749-7759.
Shelef LA (1983). Antimicrobial effect of spices. J Food Saf., 6: 29-44.
Smith-Palmer A, Stewart J, Fyfe L (1998). Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. Lett Appl. Microbiol., 26: 118-122.
Sørum H, L'Abée-Lund TM (2002). Antibiotic resistance in food-related bacteria - a result of interfering with the global web of bacterial genetics. Int. J. Food. Microbiol., 78: 43-56.
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.
Turker H, Birinci Yıldırım A, Pehlivan Karakaş F (2009). Sensitivity of Bacteria Isolated from Fish to Some Medicinal Plants. Turkish J. Fish Aquatic Sci., 9: 181-186.
Van Hijum SAFT, Baerends RJS, Zomer AL, Karsens HA, Martín-Requena V, Trelles O, Kok J, Kuipers OP (2008). Supervised Lowess normalization of comparative genome hybridization data-application to lactococcal strain comparisons. BMC Bioinfo., 9: 93.
Vendrell D, Balca´zar JL, Ruiz-Zarzuela I, de Blas I, Girone´s O, Mu´zquiz JL (2006). *Lactococcus garvieae* in fish: a review. Comp. Immunol. Microbiol. Infect. Dis., 29: 177-198.
Verschwele A (2005). Weed control with herbicides -opportunities and risks for the Ecological Farming. In: Abstract of the 8th Scientific Conference on Organic Farming, Kassel, Germany, 1-4 March 2005.
Wang CY, Shie HS, Chen SC, Huang JP, Hsieh IC, Wen MS, Lin FC, Wu D (2007). *Lactococcus garvieae* infections in humans: possible association with aquaculture outbreaks. Int. J. Clin. Pract., 61: 68-73.
Zargari A (1989-1992). Medicinal plants. Tehran University Press, Tehran, Iran (in Persian), Vol. 1-6.
Zhang L, Reddi U, Srinivasan U, Li S, Borchardt SM, Pillai P, Mehta P, Styka AN, DeBusscher J, Marrs CF, Foxman B (2008). Combining microarray technology and molecular epidemiology to identify genes associated with invasive group B Streptococcus. Interdiscip Perspect. Infect. Dis., 31: 47-62.