TÜBİTAK

Turk J Biol
33 (2009) 73-78
© TÜBİTAK
doi:10.3906/biy-0805-18

Antibacterial Activities of Extracts from Some Turkish Endemic Plants on Common Fish Pathogens

Hakan TÜRKER, Arzu BİRİNCİ YILDIRIM, Fatma PEHLİVAN KARAKAŞ, Hilal KÖYLÜOĞLU

Department of Biology, Abant İzzet Baysal University, Bolu - TURKEY

Received: 13.05.2008

Abstract: Antibacterial activities of 24 alcoholic and aqueous extracts from 8 endemic Turkish plants (*Crocus abantensis*, *Crocus ancyrensis*, *Galanthus plicatus* subsp. *byzantinus*, *Paronychia chionaea*, *Astragalus gymnolobus*, *Trifolium pannonicum* subsp.*elongatum*, *Eryngium bithynicum,* and *Convolvulus galaticus*) of 7 different families were screened. Antibacterial activity was carried out with 5 different fish pathogens (*Aeromonas hydrophila, Yersinia ruckeri, Streptococcus agalactia, Lactococcus garvieae,* and *Enterococcus faecalis*). Crude extracts of endemic plant extracts were applied against the bacteria using the disc diffusion method under in vitro conditions. *A. hydrophila* was the only inhibited bacteria from all alcoholic and aqueous extracts of *C. ancyrensis, G. plicatus, T. pannonicum, P. chionaea,* and *A. gymnolobus*. The alcoholic extracts of *T. pannonicum* among all of the plant extracts showed a broad antibacterial spectrum against *A. hydrophila, Y. ruckeri*, *S. agalactia,* and *L. garvieae* except *E. faecalis*. Among the studied endemic plants, *T. pannonicum* is a promising source for natural compounds having antimicrobial activity on fish pathogens.

Key Words: Antibacterial, antimicrobial, endemic plants, fish pathogens

Türkiye'de Bulunan Bazı Endemik Bitki Türlerinden Elde Edilmiş Ekstraktların Balık Patojenleri Üzerindeki Antibakteriyel Etkilerinin Belirlenmesi

Özet: Türkiye'ye özgü 7 farklı familyaya ait 8 adet endemik bitkiden (*Crocus abantensis*, *Crocus ancyrensis*, *Galanthus plicatus* subsp. *byzantinus*, *Paronychia chionaea*, *Astragalus gymnolobus*, *Trifolium pannonicum* subsp.*elongatum*, *Eryngium bithynicum* ve *Convolvulus galaticus*) elde edilen 24 adet alkol ve su özütlerinin antibakteriyel aktivitesi araştırılmıştır. Antibakteriyel aktivite 5 farklı balık patojeni (*Aeromonas hydrophila, Yersinia ruckeri, Streptococcus agalactia, Lactococcus garvieae* and *Enterococcus faecalis*) ile yapılmıştır. Bakterilere karşı endemik bitkilerin kaba özütleri disk difüzyon metoduna göre in vitro şartlarda uygulanmıştır. Sadece *A. hydrophila* bakterisini, *C. ancyrensis, G. plicatus, T. pannonicum, P. chionaea* ve *A. gymnolobus*' un alkolik ve su özütleri inhibe etmiştir. Bütün bitkilerden elde edilen özütler arasından *T. pannonicum* 'un alkolik özütleri *E. faecalis* hariç, *A. hydrophila, Y. ruckeri*, *S. agalactia* ve *L. garvieae* bakterileri üzerinde geniş spektrumlu antibakteriyel aktivite göstermiştir. Çalışılan endemik bitkiler arasında içerisinde doğal antimikrobiyel bileşik içeren *T. pannonicum* göz önüne alınacak en umut verici kaynaktır.

Anahtar Sözcükler: Antibakteriyel, antimikrobiyel, endemik bitkiler, balık patojenleri

Introduction

The incidence of infectious diseases in aquaculture leads to significant economic losses causing significant problems in the development of the sector (1). Various antimicrobial agents have been used for the treatment of these diseases. However, the use of antimicrobial agents in aquaculture has resulted in the development of more resistant bacterial strains (2,3). In addition, continuous use of synthetic antibiotics poses a threat to consumer health, non-target organisms, and the environment (4,5). The medicinal plants have been widely used for the treatment of common animal and human infectious diseases since antiquity (6). Therefore, the treatment of bacterial fish diseases with natural products might be safe for all organisms involved.

Increased expectations of consumers for fish meat quality and environmental and animal welfare issues are pushing the intensification of aquaculture industry causing serious outbreaks of diseases and leading to the use of antibiotics (7). Hence, new alternative natural medicines should be developed to treat and control bacterial fish diseases.

Turkish aquaculture industry has a short, but productive, history of maintaining aquaculture products. Total production of aquaculture in Turkey increased from 1990 to 2007 and reached to 140.000 t in 2007 (8). Rainbow trout (in freshwater) and European sea bass and gilthead sea bream (in saltwater) are the major species in intensive farming systems in Turkey.

Turkey has a rich plant diversity and higher endemism ratio compared to Europe. According to a recent record (9), there are 8988 native plant species in Turkey, and endemism ratio is about 33.3% with 2991 endemic plant species. Extraordinary variety of habitats and ecosystems in Turkey are a result of various climates, geomorphology and topographic structures of Anatolia, and being located in the intersection of 3 phytogeographical regions (Euro-Siberian, Mediterranean, and Irano-Turanian) (10). Scientific verification of biological activities of endemic plants may be important to screen the potential value of Turkish endemics.

There are many reports on the antibacterial activities of Turkish plants against either animal or human pathogens. However, the inhibitory activity of Turkish endemic plants has not been evaluated against any fish pathogens. To our knowledge, the present study describes, for the first time, antibacterial activities of 8 endemic plants found in Bolu against commonly occurring fish pathogens in aquaculture.

Materials and Methods

Plant material and extraction

Five endemic plant species (*Crocus abantensis*, *Crocus ancyrensis*, *Galanthus plicatus* subsp*. byzantinus*, *Paronychia chionaea,* and *Astragalus gymnolobus*) were collected from Abant Lake, Bolu, Turkey and 3 endemic species (*Trifolium pannonicum* subsp.*elongatum*, *Eryngium bithynicum* and *Convolvulus galaticus*) were collected from Gölköy, Bolu, Turkey. Identification of species was performed using "Flora of Turkey and the East Aegean Islands" (11) and voucher specimens were deposited at the Abant Izzet Baysal University (AIBU) Herbarium, Bolu, Turkey. All plant samples and collection numbers are presented in Table 1.

All collected plants were oven dried at 40 °C for a week and extracted with different solvents [Water, Methanol (MeOH), and Ethanol (EtOH)]. For aqueous extraction, 20 g from each powdered plant sample were extracted with 200 ml water at 80 °C in a water bath for 12 h and then filtered. Water was evaporated using a lyophilizator. For alcoholic extraction (MeOH and EtOH), 20 g of plant sample were Soxhlet extracted with 350 ml MeOH or EtOH at 60 °C for 12 h and the liquid portion was evaporated by a rotary evaporator. For antibacterial assay, each residue was dissolved in sterile distilled water in order to obtain a final concentration of 100 mg/ml. Plant materials, designation of treatments, and yield (%) for each extraction are summarized in Table 1.

Antibacterial assay

The disc diffusion assay (Kirby-Bauer Method) was used to screen for the antibacterial activity (12). The microorganisms used were: *Aeromonas hydrophila* and *Yersinia ruckeri,* which are gram-negative bacteria, and *Streptococcus agalactia*, *Lactococcus garvieae,* and *Enterococcus faecalis,* which are gram-positive bacteria. *A. hydrophila* (ATCC 19570) and *S. agalactia* (Pasteur Institute 55118) were purchased from Refik Saydam Hygiene Center Culture Collection. *Y. ruckeri* and *L. garvieae* were provided by Dr. Altınok, Sürmene Faculty of Marine Science, Karadeniz Technical University, Trabzon, Turkey and *E. faecalis* by Dr. Koyuncu, Faculty of Fisheries, Mersin University, Mersin, Turkey.

Pure culture of each bacterial strain was grown on Tryptic Soy Agar (TSA) and incubated for 2 days at 37 °C and 4-5 loops from each strand was transferred into culture tube containing 5 ml sterile Tryptic Soy Broth (TSB). They were incubated for 12 h at 37 °C. Mueller Hinton agar plates were inoculated with a microorganism suspension at a density of 10^6 cells/ml using cotton swabs. All extracts were sterilized by filtering through a 0.22 mm filter (Millipore) and sterile filter paper discs (Glass Microfibre filters, Whatman[â]; 6 mm in diameter) were impregnated with the extract (50 ml). There were 4 replicates in each plate and 2 plates for each extract

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

Family and plants species	Turkish name	Collection number	Part used	Extract	Designation	Yield (%)*
IRIDACEAE						
Crocus abantensis T.Baytop & Mathew	Abant çiğdemi	AUT-2010	Aerial	Water	Ex 1a	19.6
				MeOH	Ex 1b	61.4
				EtOH	Ex 1c	21.7
Crocus ancyrensis (Herbert) Maw	Ankara çiğdemi	AUT-2011	Aerial	Water	Ex 2a	20.4
				MeOH	Ex 2b	36.3
				EtOH	Ex 2c	33.6
AMARYLLIDACEAE						
Galanthus plicatus Bieb. subsp. *byzantinus* (Baker) D.A. Webb.	Kardelen	AUT-2012	Aerial	Water	Ex 3a	26.5
				MeOH	Ex 3b	16.6
				EtOH	Ex 3c	16.9
CONVOLVULACEAE						
Convolvulus galaticus Rostan ex Choisy	Sarmaşık	AUT-2013	Aerial	Water	Ex 4a	25.6
				MeOH	Ex 4b	10.0
				EtOH	Ex 4c	6.7
FABACEAE						
Trifolium pannonicum Jacq. subsp. *elongatum* (Willd.) Zoh.	Yonca	AUT-2014	Aerial	Water	Ex 5a	19.5
				MeOH	Ex 5b	9.4
				EtOH	Ex 5c	5.4
APIACEAE						
Eryngium bithynicum Boiss	Boğa dikeni	AUT-2015	Aerial	Water	Ex 6a	17.2
				MeOH	Ex 6b	12.2
				EtOH	Ex 6c	11.3
ILLECEBRACEAE						
Paronychia chionaea Boiss	Dolama otu	AUT-2016	Aerial	Water	Ex 7a	23.7
				MeOH	Ex 7b	10.0
				EtOH	Ex 7c	39.1
FABACEAE						
Astragalus gymnolobus Fischer	Geven	AUT-2017	Aerial	Water	Ex 8a	26.2
				MeOH	Ex 8b	16.0
				EtOH	Ex 8c	14.0

*Yield (%) = Weight of extract (g) / 20 g of powdered plant sample * 100

tested for each bacterium. Positive controls consisted of 5 different antimicrobial susceptibility test discs (Bioanalyse[a]): Furazolidone (100 µg), Oxytetracycline (30 µg), Tetracycline (30 µg), Erytromycin (15 µg), and Trimethoprim/sulfamethoxazole (1.25/23.75 µg). Four antibiotic discs were used for each plate and run in duplicate. Negative control consisted of distilled water.

Inoculated plates with disks were placed in a 37 °C incubator. After 16 to 18 h of incubation, inhibition zone diameter (mm) was measured. Experiments were repeated 3 times. All data were analyzed by analysis of variance (ANOVA) and mean values were compared with Duncan's Multiple Range Tests using SPSS 15 (SPSS Inc., Chicago, IL, USA).

Results and Discussion

In this study, 24 crude extracts of 8 endemic plants were screened for antibacterial activity against 5 fish pathogens. Those bacterial pathogens are the ones that commonly occur in aquaculture sector and cause serious infectious diseases and mortality in fish (13) (Table 2).

In regard to our extraction procedure, heat-stable and water-soluble antibacterial compounds were tested in this study. The methanol and ethanol extracts of *T. pannonicum* exhibited of a broad-spectrum activity against both gram-positive (*S. agalactia* and *L. garvieae*) and gram-negative bacteria (*A. hydrophila* and *Y. ruckeri*). *E. faecalis,* however, was not inhibited by any alcoholic and aqueous extracts of *T. pannonicum*. Ethanol extract of *T. pannonicum* had the highest inhibition zone (>14 mm) against *Y. ruckeri* among the tested bacteria (Table 3).

A. hydrophila was the only bacterial fish pathogen that was inhibited by both alcoholic and aqueous plant extracts of *C. ancyrensis, G. plicatus, T. pannonicum, P. chionaea,* and *A. gymnolobus* (Table 3).

C. abantensis did not show inhibitory activity against any of the fish pathogens. Similarly, Ilcim et al. (14) reported that *C. chrysanthus* was not active against their bacteria species (*Bacillus megaterium*, *B. brevis*, *Klebsiella pneumoniae*, *Escherichia coli*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Listeria monocytogenes*). However, *C. ancyrensis* showed just a little activity (<8 mm) against only *A. hydrophila.*

E. bithynicum did not show any inhibition against fish pathogens in our study, although antibacterial activity of *E. foetidium* against *Helicobacter pylori* was recorded (15). *C. galaticus* was the other plant that did not present activity against the fish pathogens used (Table 3).

All extracts of *P. chionaea* displayed inhibition against only *A. hydrophila* in our experiment. Inhibitory effect of *P. argentea* was also found against *B. subtilis*, *S. aureus*, *E. coli,* and *P. aeruginosa* (16) (Table 3).

Gram-positive bacteria commonly appear to be more susceptible to the inhibitory effects of the plant extracts compared to gram-negative bacteria. Susceptibility of gram-positive bacteria may be the result of their cell wall structure consisting of a single layer, but the gram-negative cell wall is a multi-layered structure and it is quite complex (17). On the other hand, *A. hydrophila,* a gram-negative bacterium, appeared to be more susceptible to the plant extracts used in our experiments (Table 3). Moreover, *E. faecalis,* a gram-positive bacterium, was not vulnerable to all plant extracts used.

Table 2. The most common bacterial pathogens in aquaculture (13).

Pathogen	Disease	Signs of Disease	Host
Aeromonas hydrophila	Haemorrhagic septicaemia, peritonitis, red sore disease, fin rot, red-fin disease	Erosive or ulcerative dermal lesions, haemorrhage on fins and trunk, swelling of anus, erythema	Freshwater and ornamental fish, occasionally marine fish
Yersinia ruckeri	Enteric redmouth disease, yersiniosis	Reddening of throat and mouth, hemorrhages on gills, fins	Salmonids, freshwater, ornamental and marine fish
Streptococcus agalactia	Streptococcosis, exophthalmia, haemorrhage	Haemorrhagic areas on body, mouth, fins	Bluefish, cultured sea bream, wild mullet, striped bass, sea trout and ornamental fish
Lactococcus garvieae	Lactococcosis, haemorrhagic septicemia, haemorrhagic enteritis, meningoencephalitis	Bilateral exophthalmia, darkening of skin, congestion of intestine, liver, kidney, spleen, brain, distended abdomen, bloody ascites fluids in peritoneal cavity	Farmed rainbow trout, ell, yellowtail, prawns, turbot, sturgeon
Enterococcus faecalis	Streptococcosis, exophthalmia, haemorrhage	Bacteria in liver and kidney, ulcers on fins	Rainbow trout, catfish, brown bullhead

Table 3. Antibacterial activity of the plant extracts used. A1: Erytromycin 15 µg; A2: Tetracycline 30 µg; A3: Oxytetracycline 30 µg; A4: Furazolidone 100 µg; A5: Trimethoprim/sulfamethoxazole 1.25/23.75 µg referred as positive controls. Means with the same letter within columns are not significantly different at P > 0.05.

Treatments	Mean diameter of inhibitory zones (mm ± SE)				
	Aeromonas hydrophila	*Yersinia ruckeri*	*Streptococcus agalactiae*	*Lactococcus garvieae*	*Enterococcus faecalis*
Ex 1a	-	-	-	-	-
Ex 1b	-	-	-	-	-
Ex 1c	-	-	-	-	-
Ex 2a	7.38 ± 0.18 j	-	-	-	-
Ex 2b	7.25 ± 0.16 j	-	-	-	-
Ex 2c	7.13 ± 0.13 j	-	-	-	-
Ex 3a	7.50 ± 0.19 j	-	-	-	-
Ex 3b	7.13 ± 0.13 j	-	-	-	-
Ex 3c	7.25 ± 0.16 j	-	-	-	-
Ex 4a	-	-	-	-	-
Ex 4b	-	-	-	-	-
Ex 4c	-	-	-	-	-
Ex 5a	8.75 ± 0.31 g*h*	-	-	-	-
Ex 5b	9.50 ± 0.19 f*g*	11.88 ± 0.39 f	10.25 ± 0.16 f	8.38 ± 0.18 g	-
Ex 5c	9.63 ± 0.38 f	14.25 ± 0.25 *d*	11.00 ± 0.27 e	9.63 ± 0.18 f	-
Ex 6a	-	-	-	-	-
Ex 6b	-	-	-	-	-
Ex 6c	-	-	-	-	-
Ex 7a	9.50 ± 0.60 f*g*	-	-	-	-
Ex 7b	8.50 ± 0.19 h*i*	-	-	-	-
Ex 7c	10.25 ± 0.31 f	-	-	-	-
Ex 8a	7.75 ± 0.16 *jj*	-	-	-	-
Ex 8b	8.50 ± 0.19 h*i*	-	-	-	-
Ex 8c	7.25 ± 0.16 j	-	-	-	-
Water	-	-	-	-	-
A 1	17.50 ± 0.42 *d*	13.00 ± 0.59 e	28.75 ± 0.56 b	27.63 ± 0.65 b	17.75 ± 0.16 b
A 2	28.38 ± 0.18 a	35.50 ± 0.42 b	29.50 ± 0.19 a	25.13 ± 0.64 *c*	15.75 ± 0.49 *d*
A 3	26.88 ± 0.64 b	35.38 ± 0.91 b	27.13 ± 0.44 *c*	28.63 ± 1.10 a	16.75 ± 0.31 *c*
A 4	13.00 ± 0.60 e	19.75 ± 0.16 *c*	-	12.50 ± 0.33 e	15.13 ± 0.35 e
A 5	25.50 ± 0.63 *c*	39.75 ± 0.16 a	21.75 ± 0.68 *d*	17.38 ± 0.18 *d*	26.00 ± 0.26 a

Positive controls (reference antibiotics) generally showed antibacterial activity to our test microorganisms. Because final concentrations of all extracts were adjusted with distilled water, it was used as a negative control and there was no inhibition with this control solvent (Table 3).

To our knowledge, only a few studies have been reported on the antimicrobial activity of various medicinal plants on fish pathogens throughout the world. For example, the water extract obtained from the bulb of *Allium sativum* on *A. hydrophila* and *P. fluorescens* and

the leaves of *Calotropis gigantea* on *Edwardsiella tarda* effectively marked inhibition zone, reported from Bangladesh (4). *A. hydrophila* and *Vibrio alginolyticus* were inhibited by some desert plants (*Hammada scoparia*, *Loranthus acacia,* and *Peganum harmala*), reported from Israel (5). Among the tested traditional Thai herbs, *Psidium guajava* and *Momordica charantia* displayed the highest antibacterial activity against *V. harveyi* and *V. parahaemolyticus* (1).

The popularity of natural substances, especially obtained from herbs, has been increasing for the past few years with the growing interest in ecological agriculture as an alternative bio-herbicides and bio-pesticides (18,19). Moreover, heavy antibiotic use in aquaculture needs to be reduced drastically and replaced with alternative natural medicine for treating fish diseases to avoid the emergence of antibiotic resistance in aquatic animal and environmental bacteria. Treatment with herbs having antibacterial activities is an economically and ecologically beneficial alternative in the aquaculture industry (5). The efficacy of 8 endemic plants in Turkey against common fish pathogens for aquaculture has been scientifically verified for the first time in Turkey.

Acknowledgements

Authors are grateful to Dr. Altınok, Sürmene Faculty of Marine Science, Karadeniz Technical University, Trabzon, Turkey and Dr. Koyuncu, Faculty of Fisheries, Mersin University, Mersin, Turkey for providing *Y. ruckeri, L. garvieae,* and *E. faecalis.* Thanks are due to Dr. Arzu Turker for her technical support.

Corresponding Author:
Hakan TÜRKER
Abant İzzet Baysal University,
Faculty of Science and Arts,
Department of Biology,
14280 Bolu - TURKEY
E-mail: h_turker@ibu.edu.tr

References

1. Direkbusarakom S, Ruangpan L, Ezura Y et al. Protective Efficacy of Clinacanthus nutans on yellow-head disease in black tiger shrimp (Penaeus monodon). Fish Pathol 33: 401–404, 1998.
2. Smith P, Hiney MP, Samuelsen OB. Bacterial resistance to antimicrobial agents used in fish farming: A critical evaluation of method and meaning. Annu Rev Fish Dis 4: 273-313, 1994.
3. Cabello FC. Heavy use of prophylactic antibiotics in aquaculture: A growing problem for human and animal health and for the environment. Environ Microbiol 8: 1137-1144, 2006.
4. Muniruzzaman M, Chowdhury MBR. Sensitivity of fish pathogenic bacteria to various medicinal herbs. Bangladesh J Vet Med 2: 75-82, 2004.
5. Abutbul MA, Golan-Goldhirsh O, Barazani R et al. Screening of desert plants for use against bacterial pathogens in fish. Isr J Aquacult-Bamid 57: 71-80, 2005.
6. Rios JL, Recio MC. Medicinal plants and antimicrobial activity. J Ethnopharmacol 100: 80-84, 2005.
7. FAO (Food and Agriculture Organization of The United Nation). National Aquaculture Sector Overview-Turkey (http://www.fao.org/fishery/countrysector/naso_turkey). Fisheries and Aquaculture Department, 2008.
8. TUİK. 2007. Türkiye İstatistik Kurumu, Su Ürünleri İstatistikleri. Ankara. (http://www.tuik.gov.tr).
9. Guner A, Ozhatay N, Ekim T et al. Flora of Turkey and East Aegean Islands (supplement 2), vol 11. Edinburgh University Press. Edinburgh; 2000.
10. Avcı M. Çeşitlilik ve Endemizm açısından Türkiye'nin Bitki Örtüsü. Coğrafya Dergisi 13: 27-55, 2005.
11. Davis PH. Flora of Turkey and the East Aegean Islands, vols. 1-9. Edinburgh University Press. Edinburgh; 1965-1985.
12. Andrews JM. BSAC standardized disc susceptibility testing method (V. 3). Journal of Antimicrobial Chemotherapy 53: 713-728, 2004.
13. Buller NB. Bacteria from fish and other aquatic animals: A practical identification manual. CABI Publishing. UK; 2004.
14. Ilcim A, Digrak M, Bagci E. The investigation of antimicrobial effect of some plant extracts. Turkish J Biol 22: 119-125, 1998.
15. Ndip RN, Tarkang AEM, Mbullah SM et al. In vitro anti-Helicobacter pylori activity of extracts of selected medicinal plants from North West Cameroon J Ethnopharmacol 114: 452-457, 2007.
16. Al-Bakri AG, Afifi FU. Evaluation of antimicrobial activity of selected plant extracts by rapid XTT colorimetry and bacterial enumeration. J Microbiol Meth 68: 19-25, 2007.
17. Essawi T, Srour M. Screening of some Palestinian medicinal plants for antibacterial activity. J Ethnopharmacol 70: 343-349, 2000.
18. Verschwele A. Weed control with herbicides – opportunities and risks for the Ecological Farming. 8th Scientific Conference on Organic Farming, Kassel, Germany: 291-294, 2005.
19. Perez EE, Lewis EE. Use of entomopathogenic nematodes and thyme oil to suppress plant-parasitic nematodes on English boxwood. Plant Dis. 90: 471-475, 2006.