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# Sensitivity of Bacteria Isolated from Fish to Some Medicinal Plants

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**Abstract**

Alcoholic and aqueous extracts from 22 species of herbs from Bolu (Turkey) were screened for antibacterial activity against *Aeromonas hydrophila*, *Yersinia ruckeri*, *Lactococcus garvieae*, *Streptococcus agalactiae* and *Enterococcus faecalis*. Extracts with various solvent of *Nuphar lutea*, *Nymphaea alba*, *Stachys annua*, *Genista lydia*, *Vinca minor*, *Fragaria vesca*, *Filipendula ulmaria*, *Helichrysum plicatum* showed the highest inhibitory activity. The ethanolic extract of *V. minor* and the alcoholic and aqueous extract of *N. lutea* displayed a broad antibacterial spectrum against the target organisms. The possible usage of herbs as an alternative to synthetic antibiotics is discussed.

*Keywords*: fish pathogens, plant extracts, antibacterial activity, alternative treatment.

## Introduction

Aquaculture has been a growing activity for the last 20 years worldwide and this impressive development has been attended by some practices potentially damaging to human and animal health (Naylor and Burke, 2005). The large-scale settings of aquatic animal husbandry have resulted in an increased antibiotic resistance in bacteria potentially pathogenic to fish and related environment (Smith *et al*., 1994; Alderman and Hastings, 1998; Petersen *et al*., 2002; Alcaide *et al*., 2005; Cabello, 2006). The continuous use of antimicrobial agents in aquaculture has resulted in more resistant bacterial strains in the aquatic environment. Continuous use of synthetic antibiotics reveals the threats to consumers and non-target organism in the environment (Muniruzzaman and Chowdhury, 2004; Abutbul *et al*., 2005). Treatments of bacterial diseases with various herbs have been safely used widely in organic agriculture, veterinary and human medicine (Direkbusarakom, 2004). Since ancient times, medicinal plants have been used for the treatment of common infectious diseases (Rios and Recio, 2005) and treatments with plants having antibacterial activity are a potentially beneficial alternative in aquaculture (Abutbul *et al*., 2005). Medicinal plants as the alternative agents are effective to treat the infectious diseases and mitigate many of side effects that are associated with synthetic antimicrobials (Punitha *et al*., 2008). In addition, plant-derived phytomedicines provide a cheaper source for treatment and greater accuracy than chemotherapeutic agents in this field (Punitha *et al*., 2008).

Among the common fish pathogens, *Aeromonas hydrophila* and *Yersinia ruckeri* as gram-negative, *Streptococcus agalactiae*, *Lactococcus garvieae* and *Enterococcus faecalis* as gram-positive bacteria cause infectious diseases. *A. hydrophila*, the most common bacterial pathogen in freshwater fish, has been recognized to be the aetiological agent of several distinct pathological conditions including tail/fin rot and haemorrhagic septicemia especially in freshwater and ornamental fish (Austin and Austin, 2007). Enteric redmouth disease mostly restricted to salmonids is caused by *Y. ruckeri* and reddening of mouth and throat is the most common symptom (Austin and Austin, 2007). *S. agalactiae*, *L. garvieae* and *E. faecalis* are closely related groups of bacteria that can cause similar diseases like streptococcosis, lactococcosis, haemorrhagic septicemia and ulcers in fins (Buller, 2004).

The ability of some herbs and seaweeds to inhibit activity of bacteria having potential interest as fish pathogens has been documented (Direkbusarakom, 2004; Muniruzzaman and Chowdhury, 2004; Abutbul *et al*., 2005; Borisutpeth *et al*., 2005; Bansemir *et al*., 2006; Dubber and Harder, 2008). Some of the local herbs and desert plants were reported to inhibit the pathogenic bacteria in aquaculture and referred to limited number of plant species (Direkbusarakom, 2004; Muniruzzaman and Chowdhury, 2004; Abutbul *et al*., 2005; Borisutpeth *et al*., 2005). However, there is limited knowledge about antimicrobial activity of herbs from Turkey as a natural treatment for fish bacterial pathogens. Therefore, the objective of the present study was to evaluate the antibacterial activity of alcoholic and aqueous extracts obtained from 22 medicinal plants in Bolu, Turkey, on most frequently isolated bacteria in aquaculture industry.

## Materials and Methods

### Plant Material and Extraction

Plants were collected from Bolu, Turkey, and they were identified according to "Flora of Turkey and the East Aegean Islands" (Davis, 1965-1985). The original specimens were deposited at the Abant Izzet Baysal University Herbarium, Bolu, Turkey. All plant samples and collection numbers are reported in Table 1.

All collected plants were oven dried at 40°C and extracted with water, methanol (MeOH) or ethanol (EtOH). For aqueous extraction, twenty grams from each powdered plant sample were extracted with 200 ml water at 80°C in a waterbath for 12 hours and then filtered. Water was evaporated using a lyophilizator. For alcoholic extractions, twenty grams of plant sample were soxhlet extracted with 350 ml MeOH or EtOH at 60°C for 12 hours and liquid portion was evaporated under vacuum. For antibacterial assay, each extract 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 the herbal extracts for antibiotic activity (Prescott *et al*., 1990). The microorganisms used were: *Aeromonas hydrophila* and *Yersinia ruckeri* which are gram-negative bacteria and *Streptococcus agalactiae*, *Lactococcus garvieae* and *Enterococcus faecalis* which are gram-positive bacteria. *A. hydrophila* (ATCC 19570) and *S. agalactiae (*Pasteur Institute 55118) were purchased from Refik Saydam Hygiene Center Culture Collection, Ankara, Turkey. *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) plates and incubated for 2 days at 37°C. 4–5 loops from each strand were transferred into culture tubes containing 5 ml sterile Tryptic Soy Broth (TSB) and were incubated for 12 hours at 37°C. Mueller Hinton agar plates were inoculated with a microorganism suspension at a density of $10^6$ cells/ml by using cotton swabs. All extracts were sterilized by filtering through a 0.22 µm filter (Millipore) and sterile filter paper discs (Glass Microfibre filters, Whatman®; 6 mm in diameter) were impregnated with 15 µl of extract. There were four replicates in each plate and two plates for each extract tested for each bacterium. Positive controls consisted of five different antimicrobial susceptibility test discs (Bioanalyse): 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. A paper disc embedded with the sterile water was used as the negative control.

The diameter of the inhibition zone (mm) was measured after 16 to 18 h of incubation at 37°C in an incubator. Inhibition zones > 11mm were stated as "strong", from 9 to 11 mm as "moderate" and < 9mm as "weak" activities.

Each antibacterial assay was performed in triplicate. One way ANOVA and Duncan test were used in order to evaluate the differences of the inhibition zones among the plant extracts.

## Results

Sixty-six crude extracts obtained by alcoholic and aqueous solvent of twenty-two plants were screened for antibiotic activity against five fish pathogens and results are summarized in Table 2. There was no inhibition zone in the negative control (water). Activity against gram-positive bacteria was less frequent than against gram-negative.

The growth of *A. hydrophila* was inhibited by the alcoholic extracts of *Filipendula ulmaria* at strong level (>11 mm), the alcoholic extracts of *Nuphar lutea*, *Nymphaea alba* and *Stachys annua*, the methanolic extract of *Phlomis pungens*, the ethanolic extract of *Genista lydia* and *Vinca minor* and the aqueous extracts of *Filipendula ulmaria* and *Helichrysum plicatum* at moderate level (9-11 mm) and the alcoholic extracts of *H. plicatum*, the methanolic extracts of *G. lydia* and *Bellis perennis* and the aqueous extract of *Fragaria vesca* at weak level (<9 mm).

The strong antimicrobial activities against *Y. ruckeri* were presented by the alcoholic extracts of *N. lutea* and *S. annua*, the methanolic extract of *N. alba*, the ethanolic extract of *G. lydia* and the aqueous extracts of *F. vesca* and *F. ulmaria*. Among the extracts, the ethanolic ones of *N. alba*, *V. minor* and *F. ulmaria* and the methanolic extracts of *Salvia tomentosa* and *F. ulmaria* demonstrated moderate and the alcoholic extracts of *H. plicatum* and the ethanolic extract of *Galium spurium* marked weak inhibiting activity against *Y. ruckeri*.

The strongest antibacterial activities among all plant species were obtained by the ethanolic extract of *H. plicatum* with inhibition zone of >13 mm against *Streptococcus agalactiae.* The extract of *N. lutea* from the all solvent types, the aqueous extract of *S. tomentosa* and the ethanolic extract of *G. lydia* inhibited *S. agalactiae* at moderate level whereas the extract of *N. alba* from the all solvent types, the alcoholic extracts of *Salvia verticillata* and the methanolic extract of *S. tomentosa* inhibited at weak level the same bacterium. Seven species: *N. lutea* (strong with water, moderate with EtOH and weak

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

| Family and Plants Species | Collection Number | Part Used | Extract | Designation | Yield (%) |
|---|---|---|---|---|---|
| **NYMPHAEACEAE** | | | | | |
| *Nuphar lutea* (L.) Sm | AUT-1931 | Leaves | Water | Ex 1a | 14,6 |
| | | | MeOH | Ex 1b | 10,7 |
| | | | EtOH | Ex 1c | 16,8 |
| *Nymphaea alba* L. | AUT-1932 | Leaves | Water | Ex 2a | 8,4 |
| | | | MeOH | Ex 2b | 9,0 |
| | | | EtOH | Ex 2c | 18,3 |
| **LAMIACEAE** | | | | | |
| *Salvia verticillata* L. | AUT-1903 | Leaves | Water | Ex 3a | 12,0 |
| subsp. *amasiaca* (Freyn & Bornm) Bornm. | | | MeOH | Ex 3b | 9,0 |
| | | | EtOH | Ex 3c | 5,0 |
| *Salvia tomentosa* Miller | AUT-1904 | Leaves | Water | Ex 4a | 3,8 |
| | | | MeOH | Ex 4b | 12,5 |
| | | | EtOH | Ex 4c | 6,3 |
| *Ajuga reptans* L. | AUT-1910 | Aerial | Water | Ex 5a | 32,0 |
| | | | MeOH | Ex 5b | 20,0 |
| | | | EtOH | Ex 5c | 6,0 |
| *Phlomis pungens* Willd. | AUT-1913 | Aerial | Water | Ex 6a | 4,4 |
| | | | MeOH | Ex 6b | 10,0 |
| | | | EtOH | Ex 6c | 4,0 |
| *Stachys annua* L. | AUT-1930 | Aerial | Water | Ex 7a | 13,5 |
| subsp. *annua* var. *annua* | | | MeOH | Ex 7b | 32,0 |
| | | | EtOH | Ex 7c | 36,8 |
| **FABACEAE** | | | | | |
| *Melilotus officinalis* (L.) Desr. | AUT-1911 | Aerial | Water | Ex 8a | 33,8 |
| | | | MeOH | Ex 8b | 20,0 |
| | | | EtOH | Ex 8c | 5,0 |
| *Galega officinalis* L. | AUT-1912 | Aerial | Water | Ex 9a | 25,8 |
| | | | MeOH | Ex 9b | 18,5 |
| | | | EtOH | Ex 9c | 8,0 |
| *Genista lydia* Boiss. | AUT-1926 | Aerial | Water | Ex 10a | *10,7* |
| var. *lydia* | | | MeOH | Ex 10b | 22,5 |
| | | | EtOH | Ex 10c | 8,2 |
| **URTICACEAE** | | | | | |
| *Urtica dioica* L. | AUT-1379 | Leaves | Water | Ex 11a | *17,0* |
| | | | MeOH | Ex 11b | 8,5 |
| | | | EtOH | Ex 11c | 7,4 |
| **PAPAVERACEAE** | | | | | |
| *Fumaria officinalis* L. | AUT-1906 | Aerial | Water | Ex 12a | 14,8 |
| | | | MeOH | Ex 12b | 20,0 |
| | | | EtOH | Ex 12c | 8,0 |
| **APOCYNACEAE** | | | | | |
| *Vinca minor* L. | AUT-1922 | Leaves | Water | Ex 13a | *20,9* |
| | | | MeOH | Ex 13b | 28,0 |
| | | | EtOH | Ex 13c | 21,0 |
| **BRASSICACEAE** | | | | | |
| *Capsella bursa-pastoris* (L.) Medik. | AUT-1924 | Aerial | Water | Ex 14a | *17,6* |
| | | | MeOH | Ex 14b | 18,0 |
| | | | EtOH | Ex 14c | 15,5 |
| **RUBIACEAE** | | | | | |
| *Galium spurium* L. | AUT-1927 | Aerial | Water | Ex 15a | *15,1* |
| | | | MeOH | Ex 15b | 15,5 |
| | | | EtOH | Ex 15c | 20,3 |
| **ROSACEAE** | | | | | |
| *Fragaria vesca* L. | AUT-1919 | Leaves | Water | Ex 16a | 14,3 |
| | | | MeOH | Ex 16b | 14,3 |
| | | | EtOH | Ex 16c | *2,9* |
| *Filipendula ulmaria* (L.) Maxim. | AUT-2001 | Leaves and flowers | Water | Ex 17a | 15,7 |
| | | | MeOH | Ex 17b | 5,8 |
| | | | EtOH | Ex 17c | 7,2 |
| **ASTERACEAE** | | | | | |
| *Helichrysum plicatum* DC. | AUT-1506 | Leaves and stems | Water | Ex 18a | 12,5 |
| subsp. *plicatum* | | | MeOH | Ex 18b | *6,1* |
| | | | EtOH | Ex 18c | 5,3 |
| *Tussilago farfara* L. | AUT-1058 | Leaves | Water | Ex 19a | 19,7 |
| | | | MeOH | Ex 19b | 13,4 |
| | | | EtOH | Ex 19c | 14,6 |
| *Cichorium intybus* L. | AUT-1908 | Aerial | Water | Ex 20a | 6,5 |
| | | | MeOH | Ex 20b | 7,5 |
| | | | EtOH | Ex 20c | 4,0 |
| *Bellis perennis* L. | AUT-1909 | Flowers | Water | Ex 21a | *25,0* |
| | | | MeOH | Ex 21b | *13,0* |
| | | | EtOH | Ex 21c | 7,0 |
| **SOLANACEAE** | | | | | |
| *Solanum dulcamara* L. | AUT-1438 | Leaves | Water | Ex 22a | 17,5 |
| | | | MeOH | Ex 22b | 16,0 |
| | | | EtOH | Ex 22c | 14,5 |

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

**Table 2.** Antibacterial activity of used plant extracts

| Treatments | Mean diameter of inhibitory zones (mm ± SE) | | | | |
|---|---|---|---|---|---|
| | *Aeromonas hydrophila* | *Yersinia ruckeri* | *Streptococcus agalactiae* | *Lactococcus garvieae* | *Enterococcus faecalis* |
| Ex 1a | - | - | 9,25 ± 0,16 *ij* | 11,6 ± 0,18 *f* | 10,75 ± 0,16 *f* |
| Ex 1b | 10,63 ± 0,18 *gh* | 12,38 ± 0,18 *e* | 10,25 ± 0,16 *g* | 9,3 ± 0,16 *hi* | - |
| Ex 1c | 10,25 ± 0,16 *h* | 11,25 ± 0,16 *gh* | 9,63 ± 0,18 *i* | 8,6 ± 0,18 *ij* | - |
| Ex 2a | - | - | 8,50 ± 0,19 *kl* | - | - |
| Ex 2b | 9,50 ± 0,19 *i* | 11,13 ± 0,13 *ghi* | 8,25 ± 0,16 *klm* | - | - |
| Ex 2c | 10,50 ± 0,19 *gh* | 10,63 ± 0,18 *ijk* | 8,13 ± 0,23 *lm* | - | - |
| Ex 3a | - | - | - | - | - |
| Ex 3b | - | - | 7,75 ± 0,16 *m* | 9,3 ± 0,16 *hi* | - |
| Ex 3c | - | - | 8,75 ± 0,16 *jk* | 9,5 ± 0,19 *gh* | - |
| Ex 4a | - | - | 9,50 ± 0,19 *i* | 8,4 ± 0,18 *jk* | - |
| Ex 4b | - | 10,13 ± 0,13 *kl* | 8,25 ± 0,16 *klm* | 8,0 ± 0,27 *jkl* | - |
| Ex 4c | - | - | - | - | - |
| Ex 5a | - | - | - | - | - |
| Ex 5b | - | - | - | - | - |
| Ex 5c | - | - | - | - | - |
| Ex 6a | - | - | - | 10,1 ± 0,13 *g* | - |
| Ex 6b | 10,75 ± 0,16 *gh* | - | - | 7,6 ± 0,18 *l* | - |
| Ex 6c | - | - | - | - | - |
| Ex 7a | - | - | - | - | - |
| Ex 7b | 9,63 ± 0,18 *i* | 12,00 ± 0,00 *ef* | - | - | - |
| Ex 7c | 9,25 ± 0,16 *ij* | 11,50 ± 0,19 *fg* | - | - | - |
| Ex 8a | - | - | - | - | - |
| Ex 8b | - | - | - | - | - |
| Ex 8c | - | - | - | - | - |
| Ex 9a | - | - | - | - | - |
| Ex 9b | - | - | - | - | - |
| Ex 9c | - | - | - | - | - |
| Ex 10a | - | - | - | - | - |
| Ex 10b | 7,25 ± 0,16 *l* | - | - | - | - |
| Ex 10c | 10,50 ± 0,19 *gh* | 12,13 ± 0,35 *e* | 9,63 ± 0,18 *i* | - | - |
| Ex 11a | - | - | - | - | - |
| Ex 11b | - | - | - | - | - |
| Ex 11c | - | - | - | - | - |
| Ex 12a | - | - | - | - | - |
| Ex 12b | - | - | - | - | - |
| Ex 12c | - | - | - | - | - |
| Ex 13a | - | - | - | - | - |
| Ex 13b | - | - | - | - | - |
| Ex 13c | 9,25 ± 0,16 *ij* | 9,63 ± 0,18 *l* | 12,25 ± 0,16 *f* | 12,0 ± 0,00 *ef* | 9,25 ± 0,16 *g* |
| Ex 14a | - | - | - | - | - |
| Ex 14b | - | - | - | - | - |
| Ex 14c | - | - | - | - | - |
| Ex 15a | - | - | - | - | - |
| Ex 15b | - | - | - | - | - |
| Ex 15c | - | 7,63 ± 0,18 *m* | - | - | - |
| Ex 16a | 8,75 ± 0,17 *jk* | 11,00 ± 0,00 *ghi* | - | - | - |
| Ex 16b | - | - | - | - | - |
| Ex 16c | - | - | - | - | - |
| Ex 17a | 9,25 ± 0,25 *ij* | 12,13 ± 0,30 *e* | - | 7,9 ± 0,13 *kl* | - |
| Ex 17b | 11,13 ± 0,30 *fg* | 10,25 ± 0,31 *jk* | - | - | - |
| Ex 17c | 11,63 ± 0,18 *f* | 10,75 ± 0,16 *hij* | - | - | - |
| Ex 18a | 10,63 ± 0,18 *gh* | - | - | - | - |
| Ex 18b | 7,50 ± 0,19 *l* | 7,25 ± 0,16 *m* | 11,88 ± 0,30 *f* | 8,3 ± 0,16 *jkl* | - |
| Ex 18c | 8,25 ± 0,16 *k* | 7,50 ± 0,19 *m* | 13,88 ± 0,69 *e* | 9,5 ± 0,19 *gh* | - |
| Ex 19a | - | - | - | - | - |
| Ex 19b | - | - | - | - | - |
| Ex 19c | - | - | - | - | - |
| Ex 20a | - | - | - | - | - |
| Ex 20b | - | | - | - | - |
| Ex 20c | - | | - | - | - |
| Ex 21a | - | - | - | - | - |
| Ex 21b | 8,50 ± 0,19 *k* | - | - | - | - |
| Ex 21c | - | - | - | - | - |
| Ex 22a | - | - | - | - | - |
| Ex 22b | - | - | - | - | - |
| Ex 22c | - | - | - | - | - |
| Water | - | - | - | - | - |
| A 1 | 17,50 ± 0,42 *d* | 13,00 ± 0,59 *d* | 28,75 ± 0,56 *b* | 27,6 ± 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* | 25,1 ± 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* | 28,6 ± 1,10 *a* | 16,75 ± 0,31 *c* |
| A 4 | 13,00 ± 0,60 *e* | 19,75 ± 0,16 *c* | - | 12,5 ± 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* | 17,4 ± 0,18 *d* | 26,00 ± 0,26 *a* |

Positive controls 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. Means with the same letter within columns are not significantly different at $P>0.05$.

with MeOH), *S. verticillata* (moderate with alcoholic extracts), *S. tomentosa* (weak with water and MeOH), *P. pungens* (moderate with water and weak with MeOH), *V. minor* (strong with EtOH), *F. ulmaria* (weak with water) and *H. plicatum* (moderate with EtOH and weak with MeOH) caused inhibition of the growth of *S. agalactiae.*

Antimicrobial effects against *E. faecalis* were

presented only by the aqueous extract of *N. lutea* and by the ethanolic extract of *V. minor* at moderate level.

The only ethanolic extract of *V. minor* exhibited of a broad-spectrum activity against both gram-positive (*S. agalactiae*, *L. garvieae* and *E. faecalis*) and gram-negative bacteria (*A. hydrophila* and *Y. ruckeri*).

*Ajuga reptans*, *Melilotus officinalis*, *Galega officinalis*, *Urtica dioica*, *Fumaria officinalis*, *Capsella bursa-pastoris*, *Tussilago farfara*, *Cichorium intybus*, *Solanum dulcamara* did not affect the growth of the studied pathogens.

Positive controls (reference antibiotics) generally showed antibacterial activity to our test microorganisms. Since 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 2).

## Discussion

The highest inhibitory activity (>11 mm) was obtained from extract of *N. lutea*, *N. alba*, *S. annua*, *G. lydia*, *F. vesca* and *F. ulmaria* which inhibited the growth of *Y. ruckeri*. Similarly, Digrak *et al*. (2001) found weak antibacterial activity of *S. annua* against some different pathogens. Antibacterial activities of *F. ulmaria* were also reported against *Staphylococcus aureus haemolyticus*, *Streptococcus pyogenes haemolyticus*, *Escherichia coli*, *Shigella flexneri*, *Klebsiella pneumonia and Bacillus subtilis* (Csedo *et al*., 1993; Rauha *et al*., 2000).

The ethanolic extract of *V. minor* and almost all types of extracts of *N. lutea* were two herbs that had wide spectrum antibacterial effect against the tested fish pathogens. Mehrabian *et al*. (1995) reported the antimicrobial effect of *V. minor* on some pathogen bacteria. The alcoholic extract of *H. plicatum* also inhibited growth of all bacteria, except *E. faecalis* in the current study. Chloroform and ethyl acetate extracts of *H. plicatum* presented antibacterial activity against *Staphylococcus aureus* (Erdogrul *et al*., 2001). *S. aureus*, *S. pyogenes* and *Staphylococcus epidermidis* were the most susceptible bacterial strains to aqueous extract of *H. plicatum* flowers and leaves (Turker and Usta, 2008)

The extracts from the *A. reptans*, *M. officinalis*, *G. officinalis*, *Urtica dioica*, *Fumaria officinalis*, *Capsella bursa-pastoris*, *Tussilago farfara*, *Cichorium intybus*, *Solanum dulcamara* had no inhibitory effect on any of the fish bacteria tested in the present study. However, results obtained with the use of herbal extracts are controversial: antibacterial activities of aqueous extract of *G. officinalis* (Pundarikakshudu *et al*., 2001), *U. dioica* (Gulcin *et al*., 2004; Turker and Usta, 2008), *F. officinalis* (Dulger and Gonuz, 2004a), *T. farfara* (Kokoska *et al*., 2002; Dulger and Gonuz, 2004a; Turker and Usta, 2008), *C. intybus* (Dulger and Gonuz, 2004b; Petrovic *et al*., 2004) and *S. dulcamara* (Bahadauria and Kumar, 2004; Turker and Usta, 2008) were reported against some human pathogens. Erdogrul (2002) found that *U. dioica* and *F. officinalis* did not show any antibacterial activity against 12 different bacterial species.

The use of alcohol as organic solvent provided a higher efficiency in extracting antimicrobial activities compared with water extraction. Some studies showed that alcoholic extraction method yielded higher antimicrobial activity than hexane, ethyl acetate and water (Rosell and Srivastava, 1987; Febles *et al*., 1995). Therefore, the use of alcoholic extracts may be suggested for the natural administration of antibiotics effective in fish disease control.

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 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 22 plants against fish pathogens and the efficacy of some herbs for the treatment of bacterial fish diseases has been scientifically verified. In summary, our results indicate that these species of herbs collected from Bolu, Turkey, present a significant antimicrobial activity against pathogenic fish bacteria. Finally, the observation that medicinal herbs of *N. lutea* and *V. minor* 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.

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