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Evaluation of Antibacterial activity of Medicinal Plants on Fish Pathogen *Aeromonas hydrophila*

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ABSTRACT:

This study evaluated the antimicrobial potency of aqueous extract of three common medicinal herbs, *Azadirachta indica* (Neem leaves), *Solanum torvum* (Sundakai fruit coat) and *Curcuma longa* (Turmeric,rhizome) against the in vitro growth of pathogenic bacterium *Aeromonas hydrophila* isolated from infected fresh-water fish*, Channa striatus* was assessed by disc diffusion assay.

Determination of minimum inhibitory concentration (MIC) for each aqueous herbal extract was done on Muller Hinton agar swabbed with 0.5 ml of overnight culture of A*. hydrophila* (10^6cfu/ml). After incubation period, zones of inhibition around herbal extracts incorporated discs were measured.

The strongest antibacterial activities among all plant species were obtained by the aqueous extract of *A. indica* with inhibition zone of 18 mm against *A. hydrophila. S. torvum* demonstrated moderate (11 mm) and *C. longa* marked weak (8 mm) inhibiting activity against *A. hydrophila*.

Keywords:
Azadirachta indica, *Solanum torvum*, *Curcuma longa, Aeromonas hydrophila,* anti-bacterial activity.

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INTRODUCTION

Aquaculture has been a growing activity for the last twenty years worldwide and this impressive development has been attended by some practices potentially damaging to 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 (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 (Muniruzzaman and Chowdhury, 2004). Treatments of bacterial diseases with various herbs have been safely used widely in organic agriculture (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). In addition, plant-derived phytomedicines provide a cheaper source for treatment and greater accuracy than chemotherapeutic agents in this field (Punitha *et al*., 2008). *Aeromonas hydrophila*, the most common bacterial pathogen in freshwater fish, has been recognized to be the aetiological agent of several distinct pathological conditions including tail rot, motile *Aeromonas* septicemia (MAS) and epizootic ulcerative syndrome (EUS) as a primary pathogen (Roberts *et al.,* 1992). EUS is a globally distributed disease and has become an epidemic affecting a wide variety of wild and cultured fish species (Roberts *et al.,* 1992) especially in Southeast Asia including Pakistan (Lilley *et al.,* 1997) and India (Vishwanath *et al.,* 1997). The ability of herbs to inhibit activity of bacteria having potential interest as fish pathogens has been documented (Bansemir *et al*., 2006; Dubber and Harder, 2008).

Therefore, the objective of the present study was to evaluate the antibacterial activity of aqueous extracts of *Azadirachta indica* (neem, leaves) *Solanum torvum* (sundakai, fruit coat) and *Curcuma longa* (turmeric, rhizome) on most frequently isolated bacterium in aquaculture industry, *A.hydrophila.*

MATERIALS AND METHODS

Plant material and extraction

A. indica, *S. torvum* and *C. longa* were collected from Agriculture college campus, Killikulam, Tirunelveli, India. They were surface-sterilized separately with 0.1% mercuric chloride (w/v) solution for 10 min and washed thoroughly in running tap water for 10 min, followed by shade drying for about 10 days until weight constancy was achieved (Hobbs,1994). Each sample was finely powdered in an electric blender. 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.

Antibacterial Assay

The disc diffusion assay (Kirby-Bauer Method) was used to screen the herbal extracts for antibiotic activity (Prescott *et al*., 1990). *Aeromonas hydrophila* isolated from infected freshwater fish, *Channa striatus* (Figure:1). Pure culture of *A. hydrophila* was grown on Tryptic Soy Agar (TSA, Hi media) plate and incubated for 2 days at 37°C. 3-4 colonies were transferred into culture tube containing 5 ml sterile Tryptic Soy Broth (TSB, Hi media) and was incubated for 24 hours at 37°C. All extracts were sterilized by filtering through a 0.22 µm filter (Millipore). To determine minimum inhibitory concentration (MIC), different concentrations of each herbal extract was prepared (10ppm, 20ppm, 30ppm, 40ppm and 50ppm) and were impregnated on 5 mm sterile Whatman No.1 filter paper disc. Then discs were placed on Muller Hinton agar (Hi-media) and plates were swabbed with 0.5 ml of overnight culture of A. *hydrophila* (10^6cfu/ml). Oxytetracycline and unloaded disc were used as a positive and negative control respectively (Lenette *et al.,* 1985). Each antibacterial assay was performed in triplicate. The diameter of the inhibition zone (mm) was measured (Jin *et al*., 1996) after 16 to 18 h of incubation at 37°C in an incubator and the MIC for each herb was observed and recorded (Table:1). Inhibition zones morethan 11mm were stated as "strong", from 9 to 11 mm as "moderate" and lessthan 9mm as "weak" activities.

RESULTS

The present study was attempted to find out the antibacterial activity of *A. indica, S. torvum* and *C. longa* at different concentrations against the fish pathogen *A. hydrophila.* The results of antibacterial activity of herbs against *A. hydrophila* are summarized in **Table 1.** *A. indica, S. torvum* and *C. longa* exhibited zone of inhibition against *A. hydrophila*. The strongest antibacterial activities among all plant species were obtained by the

Table: 1 Antibiotic sensitivity of *Aeromonas hydrophila* against Herbs (Values are mean ± SD).

Name of the herb	Concentration	*Aeromonas hydrophila* (10^6 cfu/ml)
		Zone of inhibition in mm
Azadirachta indica	10 ppm	0
	20 ppm	5 ± 0.8
	30 ppm	10 ± 1.1
	40 ppm	13 ± 0.9
	50 ppm	18 ± 1.2
Solanum torvum	10 ppm	0
	20 ppm	0
	30 ppm	4 ± 0.6
	40 ppm	7 ± 0.8
	50 ppm	11 ± 0.9
Curcuma longa	10 ppm	0
	20 ppm	0
	30 ppm	0
	40 ppm	3 ± 0.6
	50 ppm	8 ± 1.2

aqueous extract of *A .indica* with inhibition zone of 18 mm against *A. hydrophila. S. torvum* demonstrated moderate (11 mm) and *C. longa* marked weak (8 mm) inhibiting activity against *A. hydrophila*. Herbal discs of *A indica* (10ppm), *S. torvum* (10 and 20ppm) and *C. longa (*10 and 20 ppm), didn't produce zones of inhibition against *A. hydrophila.* Positive control (Oxytetracycline) showed antibacterial activity to *A. hydrophila* and there was no inhibition with negative control.

DISCUSSION

Parallel to increasing the resistance of microorganisms to the currently used antibiotics and the cost of production of synthetic compounds, pharmaceutical companies are now looking for alternatives. Medicinal plants could be one approach because most of them are safe, cost less and effective against a wide range of antibiotic resistant microorganisms. Seyyednejad *et al.,* (2008) showed that ethanolic extract of *Prunus mahaleb* inhibited the growth of various species of Gram positive and Gram negative bacteria. Aqueous extract of the neem is reportedly possessing anti-inflammatory, antimicrobial and immunomodulatory activities (Van Der Nat *et al.,* 1987). Wafaa *et al.,* (2007) reported the antibacterial and antifungal activities of the native and chemically modified extracts from neem seeds, seed-hulls and leaves.

In the present study, *A. hydrophila* showed 18 mm zone of inhibition at a concentration of 50 ppm against neem extract. Similarly, Dhayanithi *et al*., (2010) showed *Enterobacter sp* and *Escherichia coli* isolated from marine fish*, Amphiprion sebae* showed 15 mm zone of inhibition against neem extract. In the present study, *A. hydrophila* was susceptible to extract of *S. torvum* fruit coat and produced zone of inhibition of 11mm. Similarly, Sivapriya *et al*., (2010) stated that extracts of *S. torvum* fruit coat exhibited significant antibacterial activity against *Escherichia coli, Vibrio cholerae, Staphylococcus aureus, Bacillus subtilis, Salmonella typhimurium* and *Pseudomonas sp and* these extracts were as potent as standard antibiotics, chloramphenicol and streptomycin. Therefore *S. torvum* fruit coat may be yet another source of natural antibiotic. Wafaa *et al.,* (2007) confirmed that native extracts of *A. indica* leaves with concentration of 20 ppm are inhibitory to *Staphylococcus aureus, E. coli, Candida albicans, Aspergillus niger* and *Penicilium citrinum*. The aqueous *A. indica* leaf extract has been tested against *A. hydrophila* infection in common carp, *Cyprinus carpio* and the results showed that this plant could effectively control *A hydrophila* infection in *C.carpio* (Harikrishnan *et al.,* 2003). Similarly, Junaid *et al.,(2006)* evaluated antimicrobial efficacy of water, hexane and methanolic extracts of fresh leaf of *Ocimum gratissimum* against *A. hydrophila, Salmonella typhimurium, Escherichia coli, Yersinia enterocolitica,* and *Bacillus cereus* were determined using the agar gel diffusion method. Results obtained revealed that the water extracts of the fresh leaves of *O. gratissimum* was most potent, inhibiting all isolates with diameter zones of inhibition ranging from 5 mm to 18 mm, followed by hexane extract of the fresh leaves with zone ranging from 6mm to 14 mm whereas methanol extract of the fresh leaf showed no inhibitory effect on all isolates.

Amal *et al.,* (2007) evaluated the antimicrobial activity of indigenous Jordanian plant extracts of *Hypericum triquetrifolium*, *Ballota undulata*, *Ruta chalepensis*, *Ononis natrix*, *Paronychia argentea* and *Marrubium vulgare* against pathogenic bacteria, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and

Pseudomonas aeruginosa and reported that all these extracts had shown promising antimicrobial activity on all isolates. From this study, it was proved that among the three herbs, extract of *A. indica* was very effective against *A.hydrophila*. Heavy antibiotics used in aquaculture need to be reduced and replaced with alternative processes for treating fish diseases to avoid the emergence of antibiotic resistance in pathogenic and environmental bacteria (Sorum and L'Abee-Lund, 2002; Cabello, 2006). 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.

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