Antibacterial Activities of Salt Marsh Plants Against Marine Ornamental Fish Pathogens

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ABSTRACT
An ornamental fish health issue has become an urgent requirement for the sustaining growth of marine ornamental industry. Salt marshes are extremely important medicinal plant for a variety of reasons. The present investigation, five different salt marsh plants were selected (Arthrocnemum indicum, Helitriopium curassavicum, Ipomoea pes-caprae, Salicornia brachiata and Sesuvium portulacastrum) and crude extracts were prepared with three different solvents (aqueous, methanol and diethyl ether) and the crude extracts were tested against six fish bacterial pathogens isolated from marine aquarium (Escherichia coli, Aeromonas sp., Proteus sp., Pseudomonas aeruginosa, Pseudomonas fluorescence and Vibrio parahaemolyticus). The methanolic extracts of all salt marsh plants showed maximum zone of inhibition against Aeromonas sp. (14 mm) and minimum was observed in followed by moderate activity from diethyl ether extracts (8 to12 mm) and the aqueous extracts (2 to 10 mm) showed comparatively poor activity against all fish pathogens. The methanolic crude extracts of five salt marsh plants control the Aeromonas species. Methanolic extract of Helitriopium curassavicum and Salicornia brachiata were estimated significant effective antibacterial activity against all tested bacterial strains. Helitriopium curassavicum and Salicornia brachiata activity were reported relatively nearer to the tetracycline compound.

Key words: Antibacterial activity, ornamental fish, salt marsh plants, H. curassavicum

INTRODUCTION
Bacterial diseases are a serious concern in ornamental fish industry. Especially the Gram-negative species like Vibrio sp., Aeromonas sp., etc., cause some threaten diseases in ornamental fishes. Also a Gram-positive genus Streptococcus is frequently known to cause disease in ornamental fishes. Bacteria may be the primary cause of disease or they may be secondary invaders, taking advantage of a breach in the fish’s integument or the disease may be due to a compromise in its immune system. The majority of bacterial fish pathogens are natural inhabitants of the aquatic environment. Nearly every bacterial pathogens of fishes are capable of living independently away from its host.

Application of chemotherapeuticants has created problems via toxicity, resistance, residue leftover and possibly some public health and environmental consequences. Their efficacy under certain aquatic conditions (open-water systems) remain questionable and they can be costly. The use of chemicals in treating health problems has also been complicated by the misleading advice provided to the farmers by feed and chemical companies regarding the use of antibiotics and other
therapeutic drugs. Also bio-accumulation of chemotherapeutic agents in various organs of fishes was common and may become toxic. The use of antimicrobial agents in aquaculture has resulted in the development of more resistant bacterial strains (Smith et al., 1994; Cabello, 2006). Because of the side effects and the resistance gained by pathogens against certain antibiotics, recently much attention has been paid to biologically active plant extracts. In addition, continuous uses of synthetic antibiotics pose a threat to consumer health, non-target organisms and the environment (Muniruzzaman and Chowdhury, 2004; Abutbul et al., 2005). The use of plant extracts as alternative forms of medicine gained popularity in the late 1990’s (Cowan, 1999).

Salt marshes are comprised of extremely important medicinal plants. They serve as nursery grounds for many economically important fishes and shellfish (crabs, mussels and clams) and their help to fuel and food webs by recycling and exporting tremendous amounts of nutrients. Marine plants have demonstrated antibacterial, antifungal and antioxidant activity (Bernard and Pesando, 1989; Ballesteros et al., 1992; Graven et al., 1992; Gundidza, 1993; Rabe and van Staden, 1997; Burits et al., 2001; Lee et al., 2003) but its efficacy towards marine ornamental fish pathogens need to be tested. In this regard, the present study was undertaken to assess antibacterial activity from salt marsh plants against the bacteria isolated from diseased marine ornamental fishes.

MATERIALS AND METHODS
Collection and processing of salt marsh plants: Samples of Arthrocnemum indicum, Helitropium curassavicum, Salicornia brachiata, Sesuvium portulacastrum were collected in Marakkanam and the coastal sand dune Ipomoea pes-caprae was collected in Thirumullaivasal. The plant materials were washed with marine water followed by tap water for removing the attachments of unwanted particles. The plants were air dried under the shadow place. The air dried salt marsh plant materials were further dried in an incubator at 40°C for 24 h. The different dried salt marsh plants and sand dune were finally powdered by using mortar and pestle. Different parts of plants were used for crude extract preparations.

Preparation of plant crude extracts: Three different solvents (Aqueous (distilled water), Diethyl ether (C₂H₆O), Methanol (CH₃OH)) were used for preparing the crude plant extracts. Wong et al. (1994) method was adopted for crude extract preparations. About 100 g of salt marsh powders were soaked in 400 mL solvents (1:4 ratios) in sterile bottles and kept in dark room for 72 h at room temperature. Following incubation, plant extracts from different solvents were filtered through Whatman No. 1 filter papers. The extracts were condensed with the help of rotary evaporator (Lark VC-100A, China). The final condensed volumes of crude extracts were stored at 4°C in a refrigerator till antimicrobial assay is performed.

Isolation of bacterial pathogens from marine ornamental fishes: The bacteria from marine ornamental fishes were collected from Municipal Marine Aquarium positioned in Cuddalore town (latitude 11°45’14”N, longitude 79°45’44”E). The live fishes such as, Chaetodon plebeius, Gymnothurax species, Cephalopholis Formosa, Terapon jarbua, Cephalopholis miniata, Dascyllus trimaculatus were maintained in aquarium tanks were monitored constantly for bacterial infections. The infected body regions were scraped using sterilized inoculation loop and streaked in Zobell Marine Agar (HIMEDIA, Mumbai) slants. Here dead fishes were ice packed and moved to laboratory for microbiological analysis. The internal organs of dead fishes were removed using
sterilized forceps and scissor and they were homogenized with mortar under sterile condition. The homogenate was serially diluted and streaked in Zobel marine agar plates and incubated at 37°C at 48 h. After incubation the colonies were isolated and purified for further identification.

**Identification of bacteria:** The isolated bacterial colonies were identified using standard Bergey's manual and Conventional bio-chemical assay was carried out to identify the bacteria isolated from diseased ornamental fishes.

**Antibacterial activity:** Antibacterial assay was performed using disc diffusion method described by Bauer et al. (1966). Pathogenic bacterial strains isolated from the ornamental fishes were inoculated in sterile nutrient broth and incubated at 37°C for 24 h. Pathogens were swabbed on the surface of the Muller Hinton agar and discs (Whatman No.1 filter paper with 5 mm in diameter) were impregnated in the petridishes. Positive control (tetracycline) discs were placed in agar plates. The plates were incubated at 37°C for 16-18 h and the antibacterial activity was measured accordingly based on the inhibition zone around the disc impregnated with plant extracts.

**RESULTS**

There are six major bacterial pathogens namely *Aeromonas* sp., *Protease* sp., *E. coli*, *V. paraheamolyticus*, *P. aeruginosa* and *P. fluorescences* were isolated from the infected marine ornamental fishes through pour and spread plate techniques. The pathogens obtained from the infected fishes are depicted in the Table 1. The morphological and physiological characteristics of isolated bacteria were observed through microscopy and biochemical tests (Table 2).

**Table 1: Isolation of bacterial strains from marine ornamental fishes**

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th>Chaetodon plebeius species</th>
<th>Cephalopholis formosa</th>
<th>Terapon jarbua miniata</th>
<th>Cephalopholis Dascyllus trimaculatus</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aeromonas</em> colis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Aeromonas</em> sp.</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>Proteus</em> sp.</td>
<td>+</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Pseudomonas</em> aeruginosa</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>P. fluorescences</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>V. paraheamolyticus</em></td>
<td>+</td>
<td>-</td>
<td>+</td>
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</tr>
</tbody>
</table>

**Table 2: Identification of marine ornamental fish pathogens**

<table>
<thead>
<tr>
<th>Bacteria Biochemical tests</th>
<th>GS</th>
<th>ID</th>
<th>MR</th>
<th>VP</th>
<th>CAT</th>
<th>OX</th>
<th>CT</th>
<th>MT</th>
<th>NT</th>
<th>UT</th>
<th>TSI</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aeromonas</em> sp.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>AKS/AD/no H₂S</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>ACS/AD/no H₂S</td>
</tr>
<tr>
<td><em>P. fluorescences</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ACS/AB with G</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>AKS/ADK</td>
</tr>
<tr>
<td><em>Proteus</em> sp.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>AKS/ACD/H₂S</td>
</tr>
<tr>
<td><em>V. paraheamolyticus</em></td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>AKS/ACD/no H₂S</td>
</tr>
</tbody>
</table>


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Fig. 1: Antibacterial activities of salt marsh plants in methanolic extract

Antibacterial activity: The salt marsh plants were extracted into three different solvents such as methanol, diethyl ether and aqueous extracts. These extracts were tested for its antagonistic activities against the bacterial strains isolated and identified in the ornamental fish.

Methanolic extracts of salt marsh plants: The methanolic extracts of salt marsh plants showed more significant antibacterial activity against all the ornamental fish pathogens. Especially the S. brachiata and H. curassavicum showed maximum antibacterial activity against Aeromonas sp. (14 mm). S. portulacastrum showed minimum activity against Aeromonas sp. In E. coli (12 mm) A. indicum showed highest antagonistic activity and low activity was observed in S. portulacastrum. Likewise in P. aeruginosa (12) and P. fluorescences (10) the salt marsh plant I. pescaprae showed maximum zone of inhibition and minimum was recorded in S. portulacastrum (4 mm) and S. brachiata (6 mm). The methanolic extract of H. curassavicum (12 mm) and S. portulacastrum (12 mm) showed highest zone of inhibition in Proteus sp. and minimum was observed in the I. pescaprae (6 mm) and S. brachiata (6 mm). In V. parahaemolyticus, S. portulacastrum (12 mm) showed highest zone of inhibition was observed and minimum was in I. pescaprae (6 mm) (Fig.1).

Diethyl ether extract: In Aeromonas sp. Diethyl ether extract of H. curassavicum (9 mm) and I. pescaprae (9 mm) showed maximum zone of inhibition and minimum was recorded in S. brachiata (4 mm) (Fig. 2). Similarly E. coli A. indicum (10 mm), H. curassavicum (10 mm) and I. pescaprae (10 mm) showed highest antibacterial activity and lower activity was observed in S. brachiata (4 mm). H. curassavicum showed maximum zone of inhibition against P. aeruginosa (10 mm), P. fluorescences (12 mm) and minimum was recorded in S. brachiata (3 mm). Also the diethyl extract of H. curassavicum showed highest antagonistic activity in Proteus sp. and lower activity was observed in S. brachiata (4 mm). The diethyl ether extract of S. brachiata (10 mm) and S. portulacastrum (10 mm) showed moderate antibacterial activity against V. parahaemolyticus and minimum activity was showed by the plant A. indicum (4 mm). The aqueous extracts of all the salt marsh plants showed only an acceptable activity against Pseudomonas species, Proteus sp. and V. parahaemolyticus.

Aqueous extract: As compared to the other two solvent extract aqueous extract showed only acceptable antibacterial activities against ornamental fish pathogens (Fig. 3). In aqueous extract
Fig. 2: Antibacterial activities of salt marsh plants in diethyl ether extract

Fig. 3: Antibacterial activities of salt marsh plants in Aqueous extract

*I. pescaprae* (10 mm) and *A. indicum* (8 mm) showed maximum zone of inhibition against *Aeromonas* sp. and minimum observed in *S. brachiata* (2 mm). *E. coli* the two species of salt marsh plants such as *A. indicum* (10 mm) and *S. portulacastrum* (10 mm) recorded higher zone of clearance and lower was in *I. pescaprae* (6 mm) and *S. brachiata* (6 mm).

**DISCUSSION**

Bacterial diseases are the most common infectious problem of ornamental fishes and several bacterial infections are caused by Gram-negative organisms. The majority of bacterial fish pathogens are natural inhabitants of the aquatic environment, whether it is fresh and marine waters. Extrinsic stresses, including shipping, crowding, poor water quality and inadequate nutrition, may predispose an ornamental fish to bacterial diseases (Lewbart, 2001).

Marine halophytes are the specialized group of plants adopted for high saline conditions which includes mangroves, seaweeds, seagrass, salt marshes and blue green algae. They are also proven to have rich source of structurally diverse bioactive compounds with valuable pharmaceutical potential (Mayer and Hamann, 2002). In the present investigation, five different salt marsh plants (*Arthrocnemum indicum, Heliotropium cuassavicum, Ipomoea pes-caprae, Salicornia brachiata* and *Sesuvium portulacastrum*) crude extracts were prepared with three different solvents (aqueous, methanol and diethyl ether). These crude extracts were tested against six fish bacterial pathogens isolated from marine aquarium (*Escherichia coli, Aeromonas* sp., *Proteus* sp., *Pseudomonas aeruginosa, Pseudomonas fluorescence* and *Vibrio paraheamolyticus*).
Among the salt marsh plants, Heliotropium curassavicum showed considerable antibacterial activity against the fish pathogens. From the inhibition test of six pathogens, the zones were measured between 3 and 14 mm. The maximum zone of inhibition was observed with methanol extract against Aeromonas sp. and the minimum were observed with aqueous extract against Pseudomonas fluorescence strain. The higher activity of Heliotropium genus (Boraginaeae) may be due to the presence of “pyrrolizidine alkaloids” which exhibits several biological activities (Bull et al., 1968; Rizk, 1991).

Methanolic extract of salt marsh plant Ipomoea pes-caprae showed significant antibacterial activity against Aeromonas and Pseudomonas aeruginosa strains. The inhibition zone formation of Aeromonas was measured at 12 mm and the minimum activity was observed with diethyl ether extract against Pseudomonas fluorescence in the zone development of 5 mm. Arthrocnemum indicum plant extract showed significant antibacterial activity of about 12 mm zone against Aeromonas sp. and Escherichia coli strains. The minimum activity was observed with diethyl ether and aqueous extract against Proteus sp. and Vibrio paraheamolyticus pathogens. Sesuvium portulacastrum extract showed considerable zone of inhibition range between 2 to 12 mm. The maximum zones of inhibition were observed with methanol extract against Proteus sp. and Vibrio paraheamolyticus strains. The minimum zone of inhibition was observed in aqueous extract against Pseudomonas aeruginosa.

Biradar et al. (2007) reported that the alcohol extract of ten medicinal plants were better active against ornamental fish pathogen Aeromonas hydrophila collected from Carassius auratus. Turk et al. (2009) reported that Aeromonas hydrophila was inhibited by both alcoholic and aqueous extracts. Similar results were observed in the present study with Salicornia brachiata extract showed considerable antibacterial activity with all three extracts against the fish pathogen. The zone formations were measured from 2 to 14 mm. The maximum and minimum zones of inhibition were observed in methanol and aqueous extracts respectively against the Aeromonas sp.

Kumar et al. (2009) reported that the antimicrobial compounds from marine halophytes (Salicornia brachiata, Suaeda maritima and Sesuvium portulacastrum) revealed that antimicrobial activity were due to the presence of bioactive components such as sulfated polysaccharides. In addition the presence of sulfated galactose unit of the phycocolloid, 2-N-palmitoyl, 1-4-5, dihydro, 1,3,4,5 tetrahydroxy spingosine and halogenated compounds were also identified as significant antimicrobial activity (Bergold and Murphy, 1973; Mithlesh et al., 1988; Hay et al., 1988; De Nys et al., 1995). Ethanolic extracts of some coastal plants also possess activity against the pathogens. Nair and Chanda (2007) reported that the ethanolic extracts of nine medicinal plants showed significant antibacterial activity against the human pathogens than the other extracts. Boopathy (2003) studied the biology and antimicrobial activities of salt marsh and coastal plants. He examined the ethanolic extracts of Suaeda monoica and Suaeda maritima salt marsh plant showed effective antimicrobial activities towards dreadful pathogens.

Anti-microbial activity of salt marsh differs with the solvent extracts and against the pathogen; it may be due to the habitat and the season of the salt marsh collection (Majak et al., 1980). The present investigations also provide the effective activity and an alternative source of antibacterial compounds for ornamental fish pathogens. It is also quite evident to Castro et al. (2008) report. Sheela and Kannan (2003) were reported that the antimicrobial activities of plants may vary from species to species. The efficiency of the antimicrobial activity of plants should be determined by the physiological and biochemical synthesis of antimicrobial principles.
In the present study, methanolic extracts of all salt marsh plants showed superior activity (4 to 14 mm) followed by moderate activity from diethyl ether extracts (3 to 12 mm) and the aqueous extracts (2 to 10 mm) showed comparatively poor activity against all fish pathogens. Similar results were reported from salt marsh plants (Boopathy, 2003), seaweeds (Karthikaidevi et al., 2009) against human pathogens, seaweeds (Kolanjinathan et al., 2009) and mangroves (Choudhury et al., 2005) against fish pathogens. Methanolic extracts showed the potential activity against all the fish pathogens when compared to diethyl ether and aqueous extracts. Similar findings were reported by Castro et al. (2008) that methanolic extracts have a high potential and alternative source of antibacterial compounds for fish pathogens than the other extracts.

Following the methanolic extract of the salt marsh, diethyl ether also possesses persuasive activity against the pathogens. Similar trend was renowned by Nadal et al. (1966) that benzene and diethyl ether were suitable solvents for extracting the antibiotic components. Karthikaidevi et al. (2009) reported that the diethyl ether extract of seaweeds also effectively active against bacterial pathogens. Similarly, the present study diethyl ether extracts of salt marsh, Helitropium curassavicium persuasive active against the fish pathogens. Turker et al. (2009) reported that the aqueous extracts of medicinal plants were the effective against the pathogens and similar results were obtained in the present study with Ipomoea pes-caprae and Sesuvium portulacastrum showed activity against the Aeromonas sp., Escherichia coli and Proteus sp., respectively.

CONCLUSION

The present study clearly showed the methanolic extract of Helitropium curassavicium and Salicornia brachiata test results on antibacterial activity were significant against several ornamental fish pathogenic strains. Hence, further investigations on isolation and purification of the above salt marsh compounds will light up the commercialization for veterinary medicines.

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