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Antibacterial Activity of the Mangrove Leaves *Exoecaria agallocha* Against Selected Fish Pathogens

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Abstract: The antibacterial screening of chloroform extract of *Exoecaria agallocha* was studied. Chloroform extract of leaves exhibited strong inhibitory activity against all the pathogens tested. The six chloroform fractions of *E. agallocha* leaf extracts were collected and tested against the fish pathogens. The 3rd fraction of chloroform from the leaf extracts were showed maximum activity against *Bacillus subtilis*, followed by *Aeromonas hydrophyla*, *Vibrio parahaemolyticus*, *V. harveyi* and *Serratia* sp., respectively. Of these 3rd fraction showed maximum activity (12 mm) against *Vibrio parahaemolyticus,* followed by *Bacillus subtilis* (10 mm), *V. harveyi*, (10 mm) and *A. hydrophyla* (8 mm). However another chloroform fraction from *E. agallocha* showed no activity against selected fish pathogens.

Key words: Antibacterials, *Exoecaria agallocha*, fish pathogens, pichavaram

INTRODUCTION

Bacterial diseases are responsible for heavy mortality in wild and cultured fish. The problems in the farms are usually tackled by preventing disease outbreaks or by treating the actual disease with drugs or chemicals. The use of antimicrobial agent has increased significantly in aquaculture practices (Alderman and Michel, 1992). Antibiotics used in both human as well as veterinary medicines have been tried experimentally to treat bacterial infections of fish. Problems including solubility, palatability, toxicity, cost, delivery and governmental restrictions have limited the available antibiotics to a select few, especially in food fish culture. Decreased efficacy and resistance of pathogens to antibiotics has necessitated development of new alternatives (Smith *et al*., 1994).The discovery of antibiotics in the early twentieth century provided an increasingly important tool to combat bacterial diseases, as antibiotics are increasingly used and misused, the bacterial strains become resistant to antibiotics rapidly. Therefore, screening of antibacterial activity of medicinal plants are very important since vast number of medicinal plants have been used for centuries as remedies for human diseases. Mangroves and mangrove associates are widely used throughout the world. Mangroves have been a source on several bioactive compounds. Mangrove plants have been used in folklore medicines and extracts from mangrove species have proven activity against human, animal and plant pathogens. Secondary metabolites like alkoloids, phenolics, steroids, terpenoids have been characterized from mangroves and have toxicological, pharmacological and ecological importance (Bandaranayake, 2002). Scanty literature is available on the antibacterial activity of mangroves. However, studies of other biological activities in general are available. The study of Premnathan *et al.* (1992, 1996) revealed that the mangroves were found highly effective for antiviral activity as compared to seaweeds and sea grasses. Kokpal *et al.* (1990) had also reported the bioactive compounds from mangrove plants. Some mangrove plants had shown insecticidal activity (Miki *et al*., 1994, Ishibashi *et al*., 1993). Wu *et al.* (1997) reported the cytotoxic and antiplatelet aggregation activity of methanol extract of *Aglaia elliptifolia*. They provide a rich source of steroids, triterpenes, saponins, flavonoids, alkaloids and tannins (Bandaranayake, 1995). Antimicrobial activity of plant constituents such as phenol, quinines, flavones, flavor-noids, tannins, terpenoids, essential oils and alkaloids have been reported by several authors Edeoga *et al.* (2005). There is a continuous and urgent need to discover new antimicrobials with diverse chemical structures and novel mechanism of action for new and reemerging infectious diseases (Rojas *et al*., 2003). The main objectives of this study to screen the *E. agallocha* for a variety of biological activities, with the aim of identifying novel antibacterials.

MATERIALS AND METHODS

Collection and preparation of sample: Fresh elder leaves from mangrove specie *E. agallocha* from Pichavaram mangrove forest (Lat. 11º 27′ N, Long. 79º 47′ E) southeast coast of India were collected during

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Table 1: Antibacterial activity of column chromatographic fractions (1-6) of *Exoecaria agallocha* against chosen fish pathogens

Name of the fish pathogens	Zone of inhibition in mm in diameter					
	Fractions of Chloroform ($CHCl_3$)					
	1	2	3	4	5	6
A. hydrophilla	-	-	8	-	-	-
B. subtilis	-	-	10	-	-	-
Serraita sp.	-	-	-	-	-	-
V. parahemolyticus	-	-	12	-	-	-
V. harveyi	-	-	10	-	-	-

the low tide, between 18 and 21 February 2008. And thoroughly washed with sterile distilled water to remove adhering soil particles and salts. The chopped air-dried leaves (1 Kg) were taken separately in an air tight glass jars and required quantity of ethanol and water mixture (3:1 ratio) was added and kept under dark (percolation method). After 7 days, the contents were stirred well and then filtered by using muslin cloth. The plant extracts were concentrated to two third of the volume by distillation. The colloidal form of the plant extract was stored in a sterile glass container for further use.

Fractionation of bioactive compounds by Silica column chromatography: The colloidal form of extract obtained through percolation were separately suspended in water and defatted with diethyl ether (Kanchapoom *et al*., 2001). Individual aqueous layer of extracts were subjected to column chromatography packed with 500 g of silica gel (230-400 mesh) (MERCK) with the maximum height of 50 cm and eluted successively with 30 mL of n-hexane, benzene, chloroform, acetone, ethanol and water. MERCK AR grade solvents were used. The obtained fractions were labelled and stored at -80ºC (SANYO-JAPAN) for further use.

Antibacterial assay: Antibacterial assay were carried out by disc diffusion technique followed by (Bauer *et al*., 1966) was used for screening the medicinal plant extract against chosen bacterial fish pathogens viz., *B. subtilis, A. hydrophyla*, *V. parahaemolyticus* and *V. harveyi*, *Serratia* sp. Whatmann No. 1 filter paper disc (6mm diameter) impregnated with different fractions (mg/disc) were placed on Mueller Hinton agar (Himedia, Mumbai) which was previously inoculated with test organisms. Control disc was maintained without the fractions. All the plates were incubated overnight at 37ºC under static conditions. After 24 h, the zone of inhibition appearing around the discs were measured and recorded in millimeter in diameter. Triplicate samples were maintained for each bacterial strain.

RESULTS AND DISCUSSION

The *E. agallocha* leaf extracts were tested for the antimicrobial sensitivity against the fish pathogens disc diffusion assay of the six chloroform fractions of *E. agallocha* leaf extracts (Table 1).

The 3rd fraction of chloroform extract showed maximum activity (12 mm) against *V. parahaemolyticus*. The present result more or less consistent with the result of (Chandrasekaran *et al*., 2009) who reported that the range of mean zone of inhibition was between 8.3 and 22.5 mm for methanol extracts of the mangroves tested while for aqueous extracts it was between 7.3 and 18 mm. The present results showed by *B. subtilis* (10 mm), *V. harveyi,* (10 mm) and *A. hydrophyla* (8 mm). However another chloroform fraction from *E. agallocha* showed no activity against selected fish pathogens.

The *Exoecaria agallocha* is well known to contain skin irritants. In traditional Thai medicine, the bark and wood of the plant is used to treat flatulence (Karalai *et al*., 1994). In Srilanka, the smoke of the burning wood is used in the treatment of leprosy and the root, when pounded with ginger, as an embrocation for swollen hands and feet (Jayaweera, 1980). The milky latex exuded from the bark may cause blindness or blistering of the skin (Karalai *et al*., 1994). This latex has been used as a poison for fish by adding it to water and to poison arrowheads. Some triterpenoids isolated from this plant have been found to posses anti-tumor promoting activity (Konishi *et al*., 1998). The extracts of the tested salt marsh halophytes showed no antibacterial activity as these plants produced a mean zone of inhibition less than 10 mm, whereas, the extracts of mangrove plants showed significant antibacterial activity.

CONCLUSION

In recent years, screening of mangrove plants for a variety of biological activities, further attention should be paid to develop the novel drugs from natural product is prerequisite.

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