**In vitro** antibacterial activity of leaf extracts of *Rhizophora mucronata* L. against multi drug resistant *Vibrio* spp. isolated from marine water Lobster’s larvae hatcheries

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Present article consists the **in vitro** antibacterial study carried out using leaf extracts of *Rhizophora mucronata* L., a mangrove plant. Extracts were screened for antibacterial activity against multi-drug resistant *Vibrio harveyi* and *Vibrio campbellii*. The same were isolated from Lobster’s larvae hatchery water. Antibiotic resistance and sensitivity of the isolated *Vibrio* spp. were tested with Hi-Media antibiotic disc. Out of total eight antibiotics discs used, *V. harveyi* was found highly resistant to ampicillin, oxacillin, cephlothin, vancomycin, erythromycin, and clidamycin, while, it was sensitive towards chloramphenicol (19 mm) and gentamycin (9 mm). Whereas, *Vibrio campbellii* was found highly resistant to ampicillin, oxacillin, cephlothin, vancomycin, and erythromycin. *Vibrio campbellii* was highly sensitive to chloramphenicol (20 mm), and moderately sensitive to clindamycin (9 mm), and gentamycin (14 mm). Chloroform leaf extract of *Rhizophora mucronata* L. was found to be the most active one against both *Vibrio* spp., while, hexane, ethyl acetate, and methanol extract revealed moderate activity against both *Vibrio* spp. However, distilled water extract did not have any activity. Chloromphenicol from the Hi-Media antibiotic and chloroform extract from *Rhizophora mucronata* leaves can be used as a source of alternative compound to control the *Vibrio* spp. which infects the Lobster’s larvae.

[Keywords: *Vibrio* spp., Antibiotic sensitivity, *Rhizophora mucronata*, Antibacterial activity]

**Introduction**

Capture fisheries contribute equally to the fish and shellfish products as compared to aquacultured organism in India, until 1990s¹. In the field of aquaculture, both therapeutic and environmental problems have been addressed, since antimicrobial agents are released into the surrounding water during treatment of bacterial fish diseases²³. Bacteria of the genus *Vibrio* is commonly found in the coastal and estuarine waters. Some *Vibrio* strains are pathogenic and can cause Vibriosis, a serious infectious disease in both wild and cultured fish and shellfish⁴. In recent years, Vibriosis has become one of the most important bacterial diseases in maricultured organisms, affecting a large number of species of fish and shellfish⁵–⁶. Occurrence of antibiotic – resistant bacteria also increased high concentration in aquatic environments⁷. Additionally, bacteria that are pathogenic to humans may occur naturally in farmed fish or aquatic environments and make their way to humans with the spread of resistance genes, leading to health problems⁸. Progressive increase in antimicrobial resistance in enteric pathogens in developing countries is becoming a critical area of concern⁹. In the past several decades, the reliance on antibiotic aquaculture has steadily increased, and yet very little is known about the quantity of residues entering into the environment¹⁰. Extent of antibiotic use is indicative of the elution pressure exerted on bacteria⁷, and may have developed a range of efficient mechanisms to render ineffective the antibiotics used against them¹¹.

The unique family of Rhizophoraceae, salt tolerant species of mangrove tree, produce phytochemicals in their roots and leaves which prevents the growth of invasive plant and algae that are present in the immediate ecosystem. This phenomenon is termed ‘Allelopathy’ and is referred to any biochemical interaction among plants and microorganisms¹². Recently in Japan, it has been reported that a polysaccharide extract from the leaf of *Rhizophora apiculata* (designated as RAP) inhibited HIV-1 or HIV-2 or SIV strains in various cell cultures and

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assay system. Vibrio harveyi is a significant pathogenic bacterium in marine aquaculture, and recognized as the main causative agent of luminous Vibrios, which often results in mass mortality in cultured marine animals. These animals are found to be highly susceptible to this opportunistic pathogenic bacterium. Vibrio harveyi is found in almost all cultured marine fish species. Antibacterial activity against some fish pathogenic bacteria other than V. harveyi and V. campbellii using different species of mangrove was studied. Secondary metabolites like alkaloids, phenolics, steroids, terpenoids have been characterized from mangrove plant for its toxicological and pharmacological importance. Present study consists the antibiotic sensitivity of V. harveyi and V. campbellii and the antibacterial activity of Rhizophora mucronata leaf extract against multi-drug resistant V. harveyi and V. campbellii.

Materials and Methods

Water samples were collected from Central Marine Fisheries Research Institute (CMFRI), Lobster’s larvae Hatchery, Chennai, India. Isolation of bacteria from the collected sample was done by using pour plating and spread plating techniques. The cultures from the plates were purified by subculture into single identical colonies. Following standard morphological and biochemical tests, colonies were characterized on the selective agar TCBS and then confirmed for identification. The series of biochemical test included the gram staining, oxidase reaction, Vogues-Prausker, Lysine decarboxylase, arginine dihydroxylase, ornithine decarboxylase, Sucrose fermentation, maltose fermentation, D-mannitol fermentation, D-xylose fermentation, cellobiose fermentation, swamming, L-arabinose, lactose fermentation, salicin fermentation, D-mannose fermentation and salt tolerance and requirement test were performed for this purpose. Bacterial susceptibility to antimicrobial agent was performed by the disc diffusion method using guidelines established by Bauer et al. The leaves of Rhizophora mucronata were collected from the mangrove forests of South Andamans. Species was identified using the Manual of Indian Mangrove.

Leaves of the plant (1 kg) were cut, shade dried, powdered and extraction was carried out with different solvents (1:8 V/V) in the increasing order of polarity like, hexane, chloroform, ethyl acetate, methanol, and sterilized distilled water sequentially by soaking overnight at ambient temperature. The extracts were freed from solvent under reduced pressure. The residue thus obtained is finally dried under vacuum and for in vitro screening of antibiotic activity.

The antibacterial assays were done by disc-diffusion method. The extracts of leaves (500 µg/6 mm disc) were carried out against two Vibrio sp. After solvent evaporation the discs were placed on Mueller-Hinton agar medium (Hi-Media, India) previously inoculated with the overnight culture of the V. campbellii and V. harveyi. The plates were incubated for 24 to 48 hr at 37°C and the zone of inhibition was measured.

Results and Discussion

Two Vibrio spp. were isolated using TCBS agar from the water samples collected from the lobster’s larvae hatchery. The genus Vibrio is gram negative, oxidase reaction positive, and grows on TCBS agar. Besides these characteristics, they also reacted in a different manner for various tests which has been given in Table 1. According to the diagnostic scheme for Vibrio species described by Suresndran.

<table>
<thead>
<tr>
<th>Name of the test</th>
<th>V. harveyi</th>
<th>V. campbellii</th>
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<tbody>
<tr>
<td>Gram staining</td>
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<td>-</td>
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<tr>
<td>Oxidase reaction</td>
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<td>Vogues-Prausker</td>
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<td>Lysine decarboxylase</td>
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<td>Arginine dihydroxylase</td>
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<td>Ornithine decarboxylase</td>
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<td>Sucrose fermentation</td>
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<td>D-mannitol fermentation</td>
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<tr>
<td>Cellobiose fermentation</td>
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<td>Swamming</td>
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<td>L-arabinose</td>
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<td>Lactose fermentation</td>
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<td>Salicin fermentation</td>
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<td>D-xylose fermentation</td>
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<tr>
<td>Salt tolerance and requirement test</td>
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<tr>
<td>10.0% NaCl</td>
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</table>
and Gopakumar\textsuperscript{30}, \textit{V. campbellii} and \textit{V. harveyi} were classified depending on biochemical and physiological characteristics, which are also presented in Table 1. 

\textit{V. harveyi} and \textit{V. campbellii} were used to check their antibiotic sensitivity to the following antibiotics, chlorompenical, cephapenical, erythromycin, gentamycin, ampicillin, cephalothin, oxacillin, and vangomycin. \textit{V. harveyi} was highly resistant to ampicillin, cephapenical, cephalothin, vangomycin, oxacillin and erythromycin, and highly sensitive to chloromenpenical (19 mm) and moderately sensitive to gentamycin (9 mm). \textit{V. campbellii} was highly resistant to ampicillin, cephalothin, vangomycin, oxacillin, and erythromycin, and highly sensitive to chloromenpenical (20 mm) and moderately sensitive to cephapenical (9 mm) and gentamycin (14 mm) (Figs. 1 and 2).

The resistance of marine \textit{Vibrio} sp. to oxytetracyclin has been reported by Nonaka \textit{et al.} and Kim \textit{et al.}\textsuperscript{33-34}, with MIC\textsuperscript{125-800} µg ml\textsuperscript{-1}. It has also been reported that the MIC of oxytetracyclin against oxytetracyclin resistant \textit{V. harveyi} is 250 times higher than MIC of the same antibiotic against the sensitive one. These results support the current findings of using the chloromenpenical to inhibit the disease causing pathogen in lobster\textsuperscript{35}. The \textit{Vibrio} strains were more resistant to ampicillin, penicillin, streptomycin, chloromenpenical and erythromycin and they were more sensitive to bactercin, gentamycin, neomycin, oxytetracyclin and vancomycin. This reveals that various antimicrobial activities were effective \textit{in vitro} against the non-cholera Vibrios\textsuperscript{42-43}.

It has reported that phenotypic identification of fifty \textit{Vibrio} bacterial strains, isolated from diseased and healthy larvae prawn\textsuperscript{36}. Oxytetracyclin was effective against a broad range of both gram positive and gram negative bacteria, usually used as feed additive to control a natural infection in aquaculture. Hence, it has been concluded that chloromenpenical has inhibition effect on \textit{V. harveyi} and \textit{V. campbellii}. Furthermore, it is also concluded that the chloromenpenical is potential to prevent the aquaculture pathogens.

Antibacterial activity was studied from the leaves extracts of \textit{R. mucronota}. These extracts were screened for antibacterial activities against multi-drug resistant \textit{V. harveyi} and \textit{V. campbellii}, which were isolated from Lobster’s larvae hatchery water. Different solvents like chloroform, hexane, methanol, ethyl acetate and distilled water were used to obtain the extracts. Chloroform extract was found to be the most active one against both \textit{Vibrio} spp. Hexane, ethyl acetate and methanol extracts were found to be moderately active against both \textit{Vibrio} spp. The distilled water extract did not show any activity against \textit{Vibrio} spp. Results of the screening revealed that the chloroform extract and hexane extract exhibited strong antibacterial activity against the lobster’s pathogen (Fig. 3). Previous reports\textsuperscript{37-39} showed that the chloroform extracts of different plants possess strong antimicrobial activity. Moreover, the non polar extracts of mangroves have shown antibacterial activity against Lobster’s pathogens\textsuperscript{27}. Furthermore, \textit{R. mucronata} was highly potent against plant pathogens: aqueous and ethanol extracts of leaves and stem of \textit{R. mucronata}.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{fig1.png}
\caption{Antibiotic sensitivity of \textit{Vibrio campbellii} with Hi-Media antibiotic}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{fig2.png}
\caption{Antibiotic sensitivity and resistant of isolated bacteria}
\end{figure}
R. mucronata has antibiotic effect on 1

References

Commission, New Delhi for the same. The first author is grateful to University Grant Meritorious Students (RFSMS) pathogenic agents against aquaculture the leaf extract of and guinea pig ileum effect on asmogen induced contractility in isolated gastrointestinal motility test in albino mice and antidiarhoea activity in castor oil and induced methanol and water extracts were subjected to N

M were tested for their nematicidal activity against Meliodogyne javanica. Chloroform, ethyl acetate, methanol and water extracts were subjected to antidiarrohea activity in castor oil and induced gastrointestinal motility test in albino mice and effect on asmogen induced contractility in isolated guinea pig ileum. It has been concluded that R. mucronata has antibiotic effect on V. harveyi and V. campbellii. Present study elucidated that the leaf extract of R. mucronata is a great potential to develop anti-pathogenic agents against aquaculture pathogens.

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