

## *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, cephalothin, vancomycin, erythromycin, and clindamycin, while, it was sensitive towards chloramphenicol (19 mm) and gentamycin (9 mm). Whereas, *Vibrio campbellii* was found highly resistant to ampicillin, oxacillin, cephalothin, 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 mucronota* 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 aqua cultured organism in India, until 1990s<sup>1</sup>. 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 diseases<sup>2-3</sup>. 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 shellfish<sup>4</sup>. 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 shellfish<sup>5-6</sup>. Occurrence of antibiotic – resistant bacteria also increased high concentration in aquatic environments<sup>7</sup>. 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 problems<sup>8</sup>. Progressive increase in antimicrobial resistance in enteric pathogens in developing countries is becoming a critical area of concern<sup>9</sup>. 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 environment<sup>10</sup>. Extent of antibiotic use is indicative of the elution pressure exerted on bacteria<sup>7</sup>, and may have developed a range of efficient mechanisms to render ineffective the antibiotics used against them<sup>11</sup>.

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 microorganisms<sup>12</sup>. 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<sup>13</sup>. *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<sup>14</sup>. These animals are found to be highly susceptible to this opportunistic pathogenic bacterium<sup>15-21</sup>. *Vibrio harveyi* is found in almost all cultured marine fish species<sup>22-26</sup>. Antibacterial activity against some fish pathogenic bacteria other than *V. harveyi* and *V. campbellii* using different species of mangrove was studied<sup>27</sup>. Secondary metabolites like alkaloids, phenolics, steroids, terpenoids have been characterized from mangrove plant for its toxicological and pharmacological importance<sup>28-29</sup>. 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<sup>30</sup>, 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, swarming, 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*<sup>31</sup>. The leaves of *Rhizophora mucronata* were collected from the mangrove forests of South Andamans. Species was identified using the Manual of Indian Mangrove<sup>32</sup>.

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<sup>27</sup>.

The antibacterial assays were done by disc-diffusion method<sup>31</sup>. 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 1—Results of the biochemical tests with the bacterial isolates from lobster's larvae hatchery water

Name of the test	Name of the organisms	
	<i>V. harveyi</i>	<i>V. campbellii</i>
Gram staining	-	-
Oxidase reaction	+	+
Vogues-Prausker	+	+
Lysine decarboxylase	+	+
Arginine dihydroxylase	-	-
Ornithine decarboxylase	+	-
Sucrose fermentation	+	-
D-mannitol fermentation	+	+
Cellobiose fermentation	+	+
Swarming	+	-
L-arabinose	-	-
Lactose fermentation	-	-
Salicin fermentation	-	-
D-mannose fermentation	+	+
D-xylose fermentation	-	-
Maltose fermentation	+	+
Salt tolerance and requirement test		
0% NaCl	-	-
0.1% NaCl	-	-
0.3% NaCl	-	-
0.5% NaCl	+	+
1.0% NaCl	+	+
3.0% NaCl	+	+
6.0% NaCl	+	+
8.0% NaCl	+	-
10.0% NaCl	-	-

and Gopakumar<sup>30</sup>, *V. campbellii* and *V. harveyi* were classified depending on biochemical and physiological characteristics, which are also presented in Table 1.

*V. harveyi* and *V. campbellii* were used to check their antibiotic sensitivity to the following antibiotics, chloromphenicol, clindamycin, erythromycin, gentamycin, ampicillin, cephalothin, oxacillin, and vangomycin. *V. harveyi* was highly resistant to ampicillin, clindamycin, cephalothin, vangomycin, oxacillin and erythromycin, and highly sensitive to chloromphenicol (19 mm) and moderately sensitive to gentamycin (9 mm). *V. campbellii* was highly resistant to ampicillin, cephalothin, vangomycin, oxacillin, and erythromycin, and highly sensitive to chloramphenicol (20 mm) and moderately sensitive to clindamycin (9 mm) and gentamycin (14 mm) (Figs. 1 and 2). The resistance of marine *Vibrio* sp. to oxytetracyclin has been reported by Nonaka *et al.* and Kim *et al.*<sup>33-34</sup>, with MIC125-800  $\mu\text{g ml}^{-1}$ . It has also been reported that the MIC of oxytetracyclin against oxytetracyclin resistant *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 chloramphenicol to inhibit the disease causing pathogen in lobster<sup>35</sup>. The *Vibrio* strains were more resistant to ampicillin, penicillin, streptomycin, chloromphenicol and erythromycin and they were more sensitive to bactericin, gentamycin, neomycin, oxytetracyclin and vancomycin. This reveals that various antimicrobial activities were effective *in vitro* against the non-cholera *Vibrios*<sup>42-43</sup>.

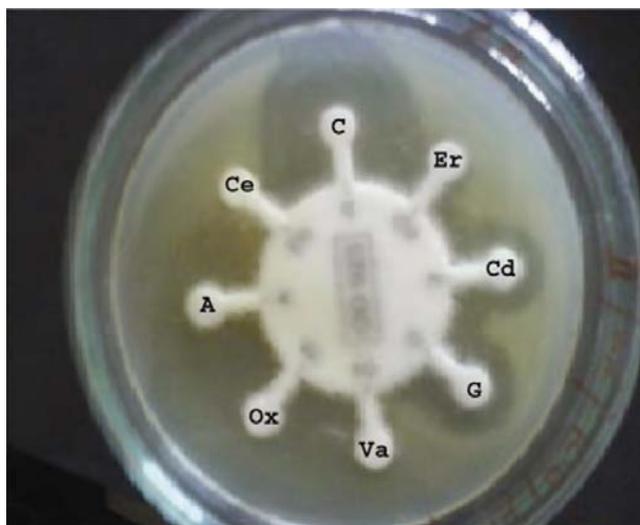


Fig.1—Antibiotic sensitivity of *Vibrio campbellii* with Hi-Media antibiotic

It has reported that phenotypic identification of fifty *Vibrio* bacterial strains, isolated from diseased and healthy larvae prawn<sup>36</sup>. 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 chloromphenicol has inhibition effect on *V. harveyi* and *V. campbellii*. Furthermore, it is also concluded that the chloromphenicol is potential to prevent the aquaculture pathogens.

Antibacterial activity was studied from the leaves extracts of *R. mucronota*. These extracts were screened for antibacterial activities against multi-drug resistant *V. harveyi* and *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 *Vibrio* spp. Hexane, ethyl acetate and methanol extracts were found to be moderately active against both *Vibrio* spp. The distilled water extract did not show any activity against *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<sup>37-39</sup> 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<sup>27</sup>. Furthermore, *R. mucronata* was highly potent against plant pathogens: aqueous and ethanol extracts of leaves and stem of *R. mucronata*

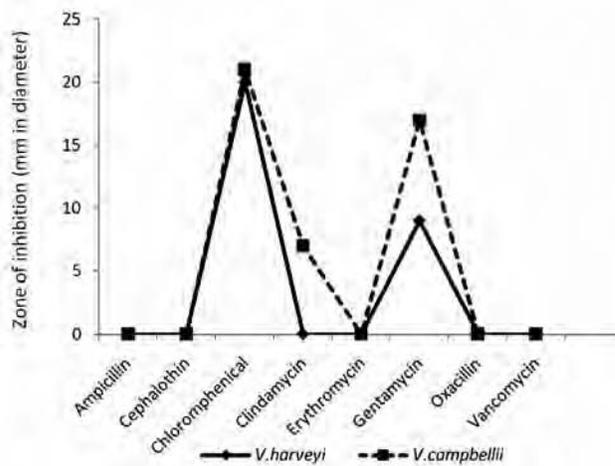


Fig.2—Antibiotic sensitivity and resistant of isolated bacteria

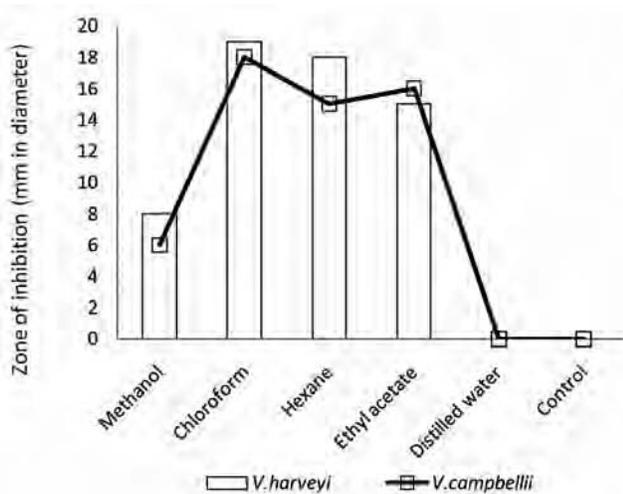


Fig.3—Antibacterial activity of *Rhizophora mucronata* leaves extract

were tested for their nematicidal activity against *Meloidogyne javanica*<sup>40</sup>. Chloroform, ethyl acetate, methanol and water extracts were subjected to antidiarrhea activity in castor oil and induced gastrointestinal motility test in albino mice and effect on asmgogen induced contractility in isolated guinea pig lieum<sup>41</sup>. 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 greta potential to develop anti-pathogenic agents against aquaculture pathogens.

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