Spanish Journal of Agricultural Research
19 (3), e0506, 12 pages (2021)
eISSN: 2171-9292
https://doi.org/10.5424/sjar/2021193-17044
Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)

RESEARCH ARTICLE

OPEN ACCESS

Effect of Asiatic mangrove plant (*Rhizophora mucronata*) extract on the growth and virulence of Vibrio harveyi causing bioluminescence disease in Penaeus monodon larviculture

Sudalayandi Kannappan[1], Krishnamoorthy Sivakumar[2], Karingalakkandy P. Jithendran[3], Balasubramanian Sivamani[4] and Peter E. Praveena[3]

[1] *Crustacean Culture Div. ICAR-Central Inst. of Brackishwater Aquac. (CIBA), Ministry Agric. & Farmer's Welf. 75 Santhome High Road, MRC Nagar, Chennai, 600 028 Tamil Nadu, India.* [2] *ICAR-Krishi Vigyan Kendra. Tamil Nadu Vet. & Anim. Sci. Univ., Kattupakkam, 603203 Tamil Nadu, India.* [3] *Aquatic Anim. Health & Environ. Div. ICAR-Central Inst. of Brackishwater Aquac. (CIBA), Ministry Agric. & Farmer's Welf. 75 Santhome High Road, MRC Nagar, Chennai, 600028 Tamil Nadu, India.* [4] *Nutr., Gen. & Biotech. Div, ICAR-Central Inst. of Brackishwater Aquac. (CIBA), Ministry Agric. & Farmer's Welf. 75 Santhome High Road, MRC Nagar, Chennai, 600028 Tamil Nadu, India.*

Abstract

Aim of study: Aim of the study: *Vibrio harveyi* bacteria are affecting shrimps during grow-out practices. The application of chemicals to control *V. harveyi* has resulted in antibiotic-resistance among bacteria. An extract of the leaves of Rhizophora mucronata was tested to control the growth and virulences of *V. harveyi.*

Area of study: This study was conducted in the Crustacean Culture Division of ICAR-CIBA, Chennai city, Tamil Nadu State, India.

Material and methods: R. mucronata plants were collected from the Pitchavaram area, and the contents extracted. The resultant extract was prepared and tested against the growth of *V. harveyi* and its virulence factors. The various functional compounds of *R. mucronata* were screened and volatile compounds were analyzed.

Main results: When *R. mucronata* extract was treated against *V. harveyi* (350 µg/mL) an inhibitory zone of 14 ± 0.1 mm was observed. At 300 µg/mL, the extract was found to be active in decreasing the luciferase to a maximum of 76 counts per second in 30 days and a similar level of bioluminescence was reduced in 15 days. During, shrimp larviculture a reduction in the cumulative percent of mortality 15.70% (p<0.033) was observed when treated with the extract of *R. mucronata.*

Research highlights: When extract (200 µg/mL) of *R. mucronata* was tested against *V. harveyi* during *Penaeus monodon* larviculture, the *V. harveyi* counts decreased (p<0.049). Volatile compounds viz, tetramethyl-6,7,8,8a-tetrahydro-5H-naphthalene-1-one (38.63%), squalene (31.19%), α-amyrin, (7.07%) and β-amyrin (8.75%) were detected. It would be desirable to use crude extracts of *R. mucronata* during shrimp culture to control *V. harveyi*.

Additional key words: antimicrobials; antagonism; shrimp larviculture; aquaculture

Abbreviations used: CFU (colony-forming unit); CPM (cumulative percent mortality); CPS (counts per second); DMSO (dimethyl sulfoxide); EPS (exopolysachharide); FT-IR (Fourier-transform infrared spectroscopy); GC-MS (gas chromatography and mass spectroscopy); IR (reflectance infrared); MIC (minimum inhibitory concentration); PL (postlarvae). PSU (practical salinity unit); SAT (salt aggregation test); VHSA (*Vibrio harveyi* selective agar)

Authors' contributions: Conceived and designed the experiment: SK and KS. Performed the work: KS, SK, BS, and PEP. Analyzed and interpreted data: KPJ, KS and SK. All authors drafted and approved the final manuscript.

Citation: Kannappan, S; Sivakumar, K; Jithendran, KP; Sivamani, B; Praveena, PE (2021). Effect of Asiatic mangrove plant (Rhizophora mucronata) extract on the growth and virulence of Vibrio harveyi causing bioluminescence disease in Penaeus monodon larviculture. Spanish Journal of Agricultural Research, Volume 19, Issue 3, e0506. https://doi.org/10.5424/sjar/2021193-17044

Supplementary material (Figs. S1 and S2) accompanies the paper on SJAR's website

Received: 27 Mar 2020. **Accepted:** 29 Jul 2021.

Funding agencies/institutions	Project / Grant
Dept. of Biotechnology (DBT), Ministry of Science and Technology, Government of India	BT/PR/13383/AAQ/03/501/2009

Competing interests: The authors have declared that no competing interests exist.

Correspondence should be addressed to Sudalayandi Kannappan: sudalikanna@gmail.com

Introduction

Vibrio is one of the virulent bacterial pathogens, responsible for causing large-scale mortality during the developmental stages of penaeid shrimps. Bioluminescence disease-causing *Vibrio harveyi* bacteria and its related species can affect almost all types of organisms (Defoirdt *et al.*, 2007) in aquaculture. The application

of antimicrobials in the aquaculture system has resulted in bacteria developing resistance to antibiotics (Igbinosa *et al.*, 2017). Edible shrimps are also perhaps a source in spreading multiple antibiotic resistances (MAR) among the bacterial pathogens to the consumers (Beshiru *et al.*, 2020). Copious technologies have been developed to control bioluminescent *V. harveyi* causing disease in the aquaculture system. "Green water culture system" is a technique used in aquaculture, wherein the shrimps or fish are co-cultured with abundant microalgae such as Chlorella. However, the micro algae production essential for aquaculture is yet to be quantified (Amir Neori, 2011). Probiotic bacteria have been used for monitoring bacterial infections and to reduce organic matter in shrimp grow-out practices, but more quantity of cells are necessary, which is cost-effective (Defoirdt *et al.*, 2008). There is an urgent need to search for other methods to control disease-causing vibrios, which is an imperative challenge for sustainable aquaculture development. It is possible that instead of chemicals, alternative bio-agents, extracted from marine plants/mangroves could be used to control resistant marine vibrios. *Rhizophora mucronata,* commonly known as Asiatic mangrove or 'red mangrove' has been reported to have healing properties against nausea, haemorrhage, and angina (Manilal *et al.*, 2015). Among the mangrove plants, *R. mucronata*, and *Avicennia marina* are known to have anti-hyperglycemic, anti-radical and anti-diabetic properties (Sur *et al.*, 2015; Aljaghthmi *et al.*, 2017) in humans.

The preliminary phytochemical analysis of *R. mucronata* revealed phenols, alkaloids, terpenoids, carbohydrates, saponins, and tannins (Chitra *et al.*, 2019). *R. mucronata* extracts at 100 µg/mL hold more potent antioxidant activity than those of *R. apiculata* and *R. annamalayana*, as evident by the presence of phenolic N–H and OH components found in mangroves leaves extract (Arulkumar *et al.*, 2020). The fruit extract of *R. stylosa* was found to control vibriosis in larvae of the mangrove crab, *Scylla serrata* (Burhanuddin *et al.*, 2019). R. mucronata extract was screened for antagonism against multi-drug resistant *V.harveyi* and *V.campbellii* (Baskaran & Mohan, 2012). The crude ethanol extract of *R. mucronata* could protect tiger shrimp from *V. harveyi* infection (Saptiani *et al.*, 2019). *R. mucronata* could be used as a bioindicator for monitoring, lead (Pb) pollution in the " Muthupet mangrove ecosystem " of Tamil Nadu, India (Ganesh Kumar *et al.*, 2019). The methanolic fraction of *R. mucronata* extract offers a substantial decrease in diabetes and metabolic impairment in rats (Adhikari *et al.*, 2016). *R. apiculata* extract improved the immune system in clownfish against infection by *V. alginolyticus* (Dhayanithi *et al.*, 2015). Therefore, an attempt was made in the present study, to evaluate *in-vitro* antagonistic effect of crude extracts of *R. mucronata* on the growth and virulence factors of *V. harveyi* for protection against bioluminescence disease in *Penaeus monodon* larviculture.

Material and methods

Isolation of *V. harveyi*

Vibrio harveyi bacterial strains were isolated from the backwaters of the Muttukadu Experimental Station (MES) of ICAR-CIBA at Chennai and identified by using various biochemical tests viz, arginine dihydrolase (-), lysine (+), ornithine decarboxylase (+), gelatinase (+), Voges-Proskauer (-), D-glucosamine (-), etc., (Abraham & Palaniappan, 2004) and compared with the characteristics of type strain *V. harveyi* ATCC 25919 as a positive control. The isolates were re-confirmed by streaking in *Vibrio harveyi* Selective agar (VHSA) medium and stored in VHSA slants at 4°C (Harris *et al.*, 1996). The pathogenicity of *V. harveyi* isolates was determined by spotting in 3% blood agar (Hi-Media, India).

A sampling of mangrove leaves and preparation of crude extracts

Leaves of *R. mucronata* (Fig. S1 [suppl]) were collected from a mangrove forest at Pichavaram in Tamil Nadu, India (Lat 11º27'N; Lon 79º47'E). The leaves were washed in a 10 mg/L solution of $KMnO_4$ for 10 min to eliminate epiphytes, sand, and other extraneous matters. The leaves were cleaned in freshwater and shade-dried at room temperature for 12 hrs. Later the leaves were pulverized by a sterile pestle and mortar and stored at -20ºC for further use. The 2.0g powder was treated with 100 mL of ethyl acetate and then extracted by the Soxhlet apparatus. The extracts were then neutralized to pH 7.0 by using 0.1 N NaOH and filtered through Whatman filter paper No.1. The extracts were later dried at 42ºC in a hot air oven for 6 hrs. For cold extraction, 1.0 g of powder was mixed with 10 mL of ethyl acetate and thereafter placed in a shaker incubator at 37°C at 50 rpm for 96 hrs. The extract was then filtered through Whatman filter paper No.1 and rotary evaporated (30°C) under vacuum and stored at 4°C. The pH was neutralized as stated earlier. The resultant extract was liquefied with 5mg/mL of 30% (v/v) dimethyl sulfoxide (DMSO) and used for testing antagonism against luminescent *V. harveyi* (Sivakumar & Kannappan, 2013).

Antibacterial assay

Antibacterial activity of the extracts was carried out by "Agar well diffusion assay" (Das *et al.*, 2005) against *V. harveyi.* Cells of *V. harveyi* (50 µL/108 CFU/mL cells

(18 hrs, old) were inoculated into Petri plates. The LB agar (35 mL) was dispensed into plates and solidified for 1 hr at room temperature. Two wells (6.0 mm) were made on the LB agar plates using a sterile steel borer. The wells were sealed at the bottom with 10 µL of 1.0% soft agar and filled with 200 µL of crude leaves extract. The plates were then incubated at 37°C for 48 hrs and zones of inhibitions on *V. harveyi* around the well were measured excluding the well. The antimicrobial activity of the *R. mucronata* extract was determined by dissolving it in 30% of DMSO in various concentrations. DMSO was used as a negative control. Similarly, the extract obtained through "cold extraction" was also tested. Each test was performed in triplicate and values were expressed as average with standard deviation.

Estimation of minimum inhibitory and minimum bactericidal concentrations (MIC & MBC)

The minimum inhibitory concentrations (MIC) for the extract were evaluated as described by Islam *et al.* (2008). Dilution methods were used to determine the MIC. In dilution tests, *V. harveyi* was tested for their ability to produce visible growth against the extracts on a series of LB agar plates against the extracts. Several concentrations of extracts (5.0 to 50 µg) were tested and the lowest concentration which inhibited the visible growth of *V. harveyi* was observed as the MIC. The plates were incubated at 37°C for 24 hrs and 20 µL of *V. harvey*i (1.8 OD or 2.19×107 CFU/mL) was confirmed for the MIC on the LB agar medium. The MBC was evaluated as the lowest concentration of a crude plant extract required to kill 99.9% of 20 µL of *V. harveyi* (1.8 OD or 2.19×107 CFU/mL).

Effects of *R. mucronata* extract on the growth and virulences of *V. harveyi*

The leaves of extract at 300 µg/mL was added to 100 mL of LB medium. Five hundred µL (24 hrs) of *V. harveyi* (1.8 OD) cells were inoculated into LB broth and incubated under shaker incubator at 37°C for 100 rpm in 5 days. Three mL of *V. harveyi* inoculum was estimated for their growth for 30 days at OD 600 nm. The growth and virulence factors such as proteolytic, lipolytic, phospholipase, thermonuclease, crude bacteriocin, exopolysaccharide (EPS), and proteases produced were estimated. Salt aggregation test (SAT) was carried out for cell surface hydrophobicity and cell adhesion was examined through bacterial adhesion to hydrocarbons test (BATH) (Soto-Rodriguez *et al.*, 2012). Each test was performed in triplicate and values were expressed on average with standard deviation.

Cell lysate preparation, estimation of luciferase, and luminescence

The luciferase enzyme produced by *V. harveyi* was tested by the luciferase kit (LUC1, Technical Bulletin MB-260, and Sigma, USA) and read by a luminometer (Victor TM X3, Perkin Elmer, USA). *V. harveyi* cells were harvested by centrifugation at 10,000 rpm for 5 min. The pellet was re-suspended in 333 µL of 1X cell lysis buffer per mL of *V. harveyi* and incubated at 25°C for 10 min. Thereafter the suspension was centrifuged at 12,000 rpm for 1 min at 4°C. The supernatant was stored in ice. Luciferase substrate (lyophilized, suspended in luciferase assay buffer) as cell lysate was equilibrated to 25°C before use. Cell lysate (20 µL) was added to 100 µL of the luciferase substrate and mixed well. Readings were recorded in 10 sec for light emission by the luminometer (Victor X3-Perkin Elmer) and expressed as counts per second (CPS, *i.e.*, photons). The light intensity was closely constant for 20 sec. The LB broth medium and 1X lysis buffer were used as a negative control for luciferase assay. For the estimation of luminescence, the *V. harveyi* cells, harvested by centrifugation at 10,000 rpm for 5 min, and its spent culture medium, were estimated for luminescence by a luminometer (Kannappan *et al.*, 2013).

Fourier transform infrared spectroscopy (FT-IR) analysis

The shade-dried powder (1.0 mg) was mixed thoroughly with 2.5 mg of dry potassium bromide (KBr) by a pestle and mortar. The powder was filled in a micro-cup (2.0 mm internal diameter) to obtain the diffuse reflectance infrared (IR) spectrum for replicate samples. All the IR spectra were recorded at 37°C in the mid-infrared range (4000-400 cm^{-1}) using Fourier Transform Infrared Spectrometer (FT-IR) Bruker IFS 66, Shimadzu. Normally, 20 scans were signal-averaged for a single spectrum. Each spectrum is displayed in terms of absorbance as calculated from the reflectance-absorbance spectrum using the Hyper-IR software (D'Souza *et al.*, 2008).

Gas chromatography and mass spectrometry analysis

Gas chromatography-mass spectrometry (GC-MS) analysis was performed by using Agilent GC-MS-5975C with the Triple-Axis Detector equipped with an auto sampler. The GC column used was fused with a silica capillary column (length 30 m × diameter 0.25 mm × film thickness 0.25 mm) with 1.51 mL helium for 1min as a carrier gas. The mass spectrometer was operated in the electron impact (EI) mode at 70 eV in the scan range of 40-700 m/z. The split ratio was adjusted to 1:10 and in-

jection volume was 1.0 µL. The injector temperature was kept at 250°C, and the oven temperature was 70°C for 3 min, which was later increased to 250°C at 14°C/min (total run time 34 min). The temperature of the transfer line and the ion source was set to a value of 230°C and the interface temperature at 240°C, the full mass data being recorded between 50-400 Daltons and scan speed 2000. Mass start time was set at 5 min and end time at 35 min. Peak of the crude *R. mucronata* extract was identified by comparison with retention times of standards and the mass spectra obtained was compared with those available in the NIST libraries (NIST 11-Mass Spectral Library 2011 version) with an acceptance criterion of a match above a critical factor of 80% (Musharraf *et al.*, 2012).

Effect of *R. mucronata* extract against *V. harveyi* during *P. monodon* larviculture

Plastic tubs were washed with 10 mg/mL of $KMnO_4$ solution (w/v) for 10 min and filled with 20 L of saline water at 20 practical salinity unit (PSU). Postlarvae (PL 10) of *P. monodon* from a private shrimp hatchery, were tested by PCR to be disease-free, following Ananda Raja *et al.* (2017), for the OIE[1] listed diseases, and were acclimatized at 20 PSU for 5 days with aeration under laboratory conditions. About 1000 PL, in the weight range of 17 to 18 ± 0.2 mg were stocked per tub. The first tub was inoculated with *V. harveyi* (10 mL of 1.8 OD) alone as a control. The second tub was considered as treatment and inoculated with *V. harveyi* (2.0g for 10 L of the extract). The third tub was considered as control, where the crude extract was added at 200 µg/mL with PL. The fourth tub was a control for PL, where neither *V. harveyi* nor extract was added. Aeration was provided for each container and the PLs were fed twice a day at 15% of their body weight. All the experimental tubs were covered on the top with a plastic lid to avoid external contamination. The water temperature, salinity, and pH were recorded once in 5 days. Experiments were carried out in triplicate. The mortality of PL was recorded daily. No water exchange was made in the containers for 30 days. The total heterotrophic and *V. harveyi* counts were enumerated (Traifalgar *et al.*, 2009) using LB agar and *V. harveyi* selective agar medium under the spread plate method (Biswas *et al.*, 2012).

Statistical analysis

The data were analyzed and expressed as means along with the standard deviation. Analysis of variance (SPSS, ver. 16.0) was carried out to find the significance ($p<0.05$) difference, if any. The cumulative percent mortality (CPM) was calculated as a Cumulative frequency/total number of observations (n) ×100.

Results

Antagonism of leaves extracts of *R. mucronata*

The results of the antimicrobial assay of crude *R. mucronata* extracts at 200, 250, 300, 350 and 400 µg/mL concentration showed 8.0 ± 0.2, 10.0 ± 0.1, 12.0 ± 0.1, 14.0 ± 0.1 and 16.0 ± 0.20 mm zones of inhibitions, respectively (excluding the well 8.0 ± 0.2 mm). As a positive control, 10 µL of oxytetracycline (250 mg/25 mL) showed a zone of inhibition of 23.0 ± 0.9 mm, whereas the DMSO as negative control showed no inhibition. The crude extract under cold extraction at concentrations of 200, 250, 300, 350 and 400 µg/mL showed 5.0 ± 0.1, 6.0 ± 0.1, 7.0 ± 0.2, 8.0 ± 0.1, 9.0 ± 0.2 mm zone of inhibitions, respectively. The MIC of crude extract at 300 µg/mL concentration showed 6.0 ± 0.10 mm against *V. harveyi* and a MBC value of 12.0 ± 0.20 mm (Fig. S2 [suppl])).

Effects of *R. mucronata* extract on growth and virulences of *V. harveyi*

The crude extract showed the maximum active antagonistic efficiency to control the growth of *V. harveyi* (OD) from the 5th day onwards. The highest OD difference was observed on the 20th day (0.37) compared to the control and the lowest on the 10th day ($p<0.05$). The bioluminescence production was decreased from 1100 to 2200 CPS against the *R. mucronata* extract for 30days as compared to control (Table 1). The maximum control on bioluminescence was detected on the 15th day (2230 CPS) and the minimum was on the 25th day (1090 CPS) ($p<0.05$). The production of intracellular luciferase by *V. harveyi* was decreased with the addition of extract from 20 to a maximum of 76 CPS in 30 days (Fig. 1). Table 1 shows that the maximum decrease in bacteriocin production (OD) by the extract was observed between the 5th and 15th days (0.39 and 0.27 respectively, $p<0.05$). The maximum decrease in protease was 0.15 OD against the extract on the 20th day. Treatment with the extract showed a weak level (+) of phospholipase, produced by *V. harveyi* as compared to very high phospholipase (++++) in the control for all the 30 days. In SAT, *V. harveyi* revealed strong hydrophobic activity from the 5th

[1] The former Office International des Epizooties (OIE) inter-governmental organization coordinating, supporting and promoting animal disease control, is now called World Organisation for Animal Health

Table 1. Effect of *R. mucronata* leaves extract against the virulence factors produced by *V. harveyi.*

Virulence factors	Days					
	5	10	15	20	25	30
Luciferase (CPS)						
Treatment	143	120	150	172	110	115
Control	182	196	188	192	140	180
Salt aggregation test (SAT)[a] (moles L^{-1})						
Treatment	1.60	1.40	1.75	1.95	2.30	2.00
Control	0.50	0.65	0.70	0.80	0.65	0.60
Bacteriocin OD at 660 nm [b]						
Treatment	2.323±0.01	2.401±0.02	2.251±0.03	2.362±0.05	2.003±0.05	1.642±0.09
Control	2.719±0.08	2.604±0.02	2.524±0.04	2.409±0.01	2.146±0.02	1.835±0.06
Protease OD at 440 nm						
Treatment	0.080±0.01	0.046±0.06	0.016±0.03	0.051±0.02	0.039±0.01	0.017±0.01
Control	0.143±0.08	0.164±0.07	0.172±0.02	0.198±0.01	0.174±0.01	0.134±0.01
Growth OD at 600 nm						
Treatment	0.683±0.02	0.649±0.01	0.688±0.03	0.363±0.01	0.742±0.02	0.617±0.01
Control	0.860±0.01	0.700±0.03	0.870±0.01	0.735±0.01	0.833±0.01	0.761±0.01
Luminescence produced (CPS)						
Treatment	82115	82200	84550	83150	85410	81120
Control	83306	83500	86780	84410	86500	82510
Phospholipase activity [c]						
Treatment	+	+	+	+	+	+
Control	+++	+++	++++	++++	++++	++++

CPS: counts per second. [a] SAT test: 0.0 to1.0 M = strongly hydrophobic; 1.0 to 2.0 M = moderately hydrophobic; 2.0 to 4.0 M = weakly hydrophobic; >4.0 M = not hydrophobic. [b] BATH test: >50% partitioning = strongly hydrophobic; 20 to 50% partitioning = moderately hydrophobic; < 20% partitioning = not hydrophobic. [c] Activity of V. harveyi: + = weak, ++ = moderate, +++ = high, ++++ = very high.

to 30th day in the control whereas, the treatment showed moderate to weak hydrophobic activities.

FT-IR analysis of *R. mucronata* extract

The FT-IR spectrum of dried leaves powder reveals various functional groups of compounds that were compared with the FTIR standard library data. The FT-IR spectrum showed the presence of significant functional groups such as aromatics, alkanes, alcohol, carboxylic acids, esters, ethers, aliphatic amines, and alkyl halides (Table 2).

GC-MS analysis of the *R. mucronata* extract

To document preliminary data on bioactive components, ethyl acetate extract was subjected to GC-MS analysis. The crude extract was found to have a mixture of secondary and volatile compounds. Fatty acid methyl esters were investigated quantitatively by GC-MS in multiple reactions monitoring the mode and thus allowing a better signal resolution without a preliminary fractionation of the extract. A total of 21 peaks were observed with retention times as presented in **Fig. 2**. The main phytoconstituent reported was tetramethyl-6,7,8,8a-tetrahydro-5H-naphthalene-1-one

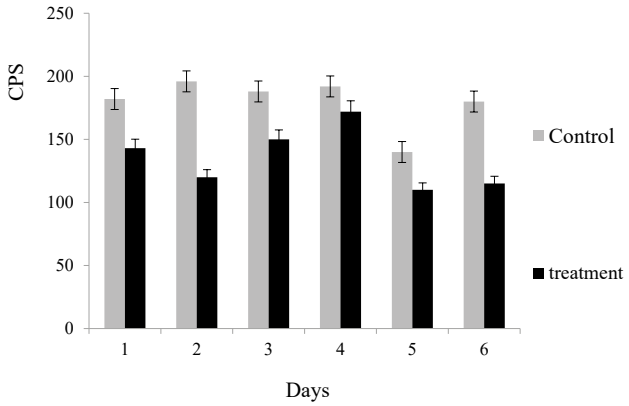

Figure 1. Effect of *R.mucronata* extract against luciferase produced by *V. harveyi*. Significant differences (p<0.033) found in the reduction of luciferase produced in treatment against the control). CPS: counts per second.

Table 2. Wave number (cm^{-1}) of dominant peaks obtained from the FT-IR absorption spectra of leaves extract of *R. mucronata.*

Virulence factors	Days					
	5	10	15	20	25	30
Luciferase (CPS)						
Treatment	143	120	150	172	110	115
Control	182	196	188	192	140	180
Salt aggregation test (SAT)[a] (moles L^{-1})						
Treatment	1.60	1.40	1.75	1.95	2.30	2.00
Control	0.50	0.65	0.70	0.80	0.65	0.60
Bacteriocin OD at 660 nm [b]						
Treatment	2.323±0.01	2.401±0.02	2.251±0.03	2.362±0.05	2.003±0.05	1.642±0.09
Control	2.719±0.08	2.604±0.02	2.524±0.04	2.409±0.01	2.146±0.02	1.835±0.06
Protease OD at 440 nm						
Treatment	0.080±0.01	0.046±0.06	0.016±0.03	0.051±0.02	0.039±0.01	0.017±0.01
Control	0.143±0.08	0.164±0.07	0.172±0.02	0.198±0.01	0.174±0.01	0.134±0.01
Growth OD at 600 nm						
Treatment	0.683±0.02	0.649±0.01	0.688±0.03	0.363±0.01	0.742±0.02	0.617±0.01
Control	0.860±0.01	0.700±0.03	0.870±0.01	0.735±0.01	0.833±0.01	0.761±0.01
Luminescence produced (CPS)						
Treatment	82115	82200	84550	83150	85410	81120
Control	83306	83500	86780	84410	86500	82510
Phospholipase activity [c]						
Treatment	+	+	+	+	+	+
Control	+++	+++	++++	++++	++++	++++

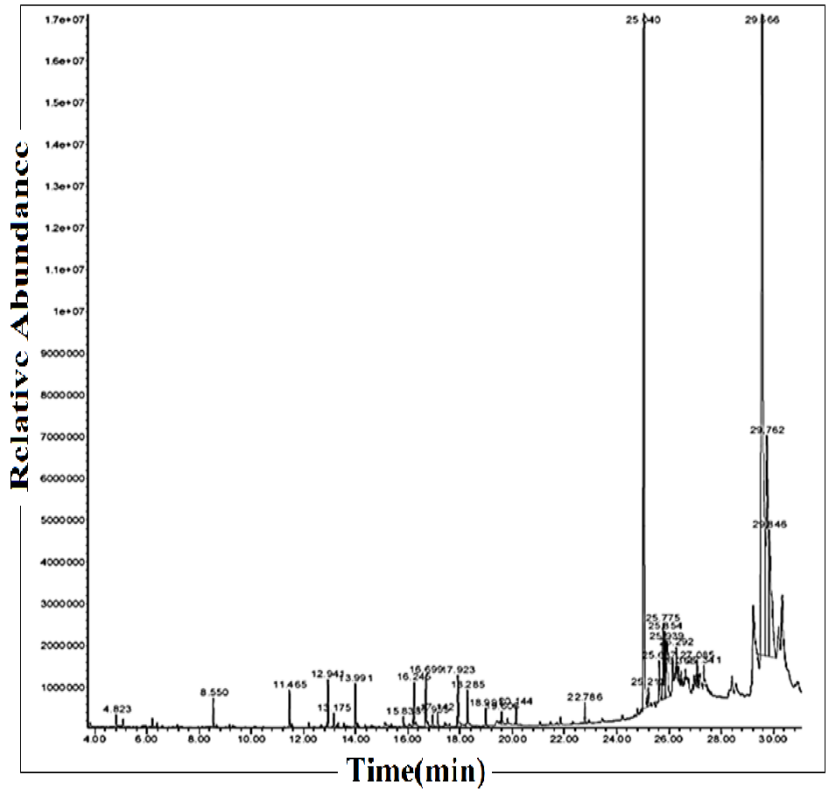

Figure 2. GC-MS profile of R. mucronata extracts showing various peaks as a mixture of volatile compounds

(38.63% peak area), followed by squalene (31.19% peak area), α-amyrin, (7.07%) and β-amyrin (8.75%). These bioactive compounds could be involved in the antagonistic activity (Table 3) against *V. harveyi* along with other compounds. Chemical constituents were identified using spectrum database NIST 11 software installed in GC-MS.

Effects of the extract of *R. mucronata* against *V. harveyi* during *Penaeus monodon* larviculture

During the larviculture of *P. monodon*, the mortality varied from 0 to 50.81% till the 30th day after treatment with the extract. The CPM increased in the control from 4% on the 5th day to 66.5% on the 30th day respectively.

Table 3. GC-MS profile of *R. mucronata* leaves extract.

Peak No.	Retention time (min)	Compound name	Peak area (%)	Molecular formula	Molecular weight (Da)
1	4.830	phenol	0.19	C_6H_6O	94.042
2	8.547	1-dodecene	0.37	$C_{12}H_{24}$	168.188
3	11.467	1-tetradecene	0.54	$C_{14}H_{28}$	196.219
4	12.933	phenol, 2,4-bis(1,1-dimethylethyl)	0.54	$C_{14}H_{22}O$	206.167
5	13.180	5,9,13-pentadecatrien-2-one, 6,10,14-trimethyl-, (E,E)-	0.20	$C_{18}H_{30}O$	262.23
6	13.994	cetene	0.63	$C_{16}H32$	224.25
7	15.838	tetradecanoic acid	0.21	$C_{14}H28O2$	228.209
8	16.245	1-nonadecene	0.68	$C_{19}H38$	266.297
9	16.695	3-eicosyne	0.89	$C_{20}H38$	278.297
0	16.956	phthalic acid, butyl tetradecyl ester	0.25	$C_{26}H42O4$	418.308
11	17.145	bicyclo[4.1.0]heptane, 3-methyl-	0.24	C_8H_{14}	110.11
12	17.929	n-hexadecanoic acid	0.89	$C_{16}H_{32}O_2$	256.24
13	18.292	5-eicosene, (E)-	0.72	$C_{20}H_{40}$	280.313
4	18.990	hexadeca-2,6,10,14-tetraen-1-ol, 3,7,11,16-tetramethyl-	0.25	$C_{20}H_{34}O$	290.261
15	19.599	7,10,13-hexadecatrienoic acid, methyl ester	0.27	$C_{17}H_{28}O_2$	264.209
16	20.137	n-nonadecanol-1	0.23	$C_{19}H_{40}O$	284.308
17	22.780	1,5,9-decatriene, 2,3,5,8-tetramethyl-	0.28	$C_{14}H_{24}$	192.188
18	25.046	squalene	31.19	$C_{30}H_{50}$	410.391
19	25.205	(e,e,e)-3,7,11,15-tetramethyl hexadeca-1,3,6,10,14-pentaene	0.34	$C_{20}H_{32}$	272.25
20	25.641	eicosane	0.58	$C_{20}H_{42}$	282.329
21	25.772	2,6,10-dodecatrien-1-ol, 3,7,11-trimethyl-, (Z,E)-	1.24	$C_{15}H_{26}O$	282.329
22	25.859	2,2,4-trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol	1.36	$C_{30}H_{52}O$	428.402
23	25.946	2-methyl-3-(3-methyl-but-2-enyl)-2-(4-methyl-pent-3-enyl)-oxetane	1.82	$C_{15}H_{26}O$	222.198
24	26.295	2,6,10,14,18-pentamethyl-2,6,10,14,18-eicosapentaene	0.61	$C_{25}H_{42}$	342.329
25	26.367	1,6,10,14,18,22-tetracosahexaen-3-ol, 2, 6, 10, 15, 19,23-hexamethyl-, (all-E)-	0.30	$C_{30}H_{50}O$	426.386
26	27.079	octacosane	0.35	$C_{28}H_{58}$	394.454
27	27.340	vitamin E	0.38	$C_{29}H_{50}O_2$	430.381
28	29.562	2,5,5,8a-tetramethyl-6,7,8,8a-tetrahydro-5h-naphthalen-1-one	38.63	$C_{14}H_{20}O$	204.151
29	29.766	beta-amyrin	8.75	$C_{30}H_{50}O$	426.386
30	29.853	alpha-amyrin	7.07	$C_{30}H_{50}O$	426.386

The difference in the overall decrease of CPM among the treatments was 12 to 16% as compared to control from 20th to 30th days. On the 30th day, a maximum reduction of 15.70% was found in the treatment, compared to control. The growth of postlarvae uniformly increased from 4-7 mg on the 10th day. The growth of *V. harveyi* when treating with the extract treatment was from 5.2×10^4 to 3.2×10^3 CFU/mL on the 30th day as compared to control (9×10^5 to 1.0×10^3 CFU/mL). In the treatment, the total heterotrophic bacterial (THB) count decreased from 4.3×10^4 CFU/mL to 1.2×10^3 CFU/mL, compared to control (6.9×10^4 to 7.2×10^3 CFU/mL) (Table 4). Significant differences were found in CPM ($p<0.033$) and *V. harveyi* counts ($p<0.049$) between the leaves extract of *R. mucronata* treated with *V. harveyi* and control.

Discussion

Mangroves are a potential source of various compounds with higher biological activity than toxicity (Manilal *et al.*, 2010). The compounds are used as antimicrobial agents and in pharmaceutical preparations. In this study, crude R. mucronota extracts showed inhibition on *V. harveyi,* but the extract obtained through the Soxhlet method gave a better inhibition than cold extraction. The crude extract obtained from the bark and collar of the Asiatic mangrove showed inhibition on *V. harveyi* (7.0 mm) and various other fish pathogens (Kesavaraju & Sreeramulu, 2017). The extract also showed anti-fungal activities on *Acremonium* sp and *Aspergillus* niger at a concentration of 75 µg/mL (Arunprabu *et al.*, 2016). Kannappan *et al.* (2018) reported the inhibition on bioluminescence disease-causing *V. harveyi* (12 mm at 350 µg/mL) by the crude extract of *R. apiculata*. The mechanism of inhibition on *V. harveyi* was due to the phytochemicals such as alcohols, phenols, alkanes, carbonyls, unsaturated aldehydes, nitro compounds, aromatics, esters, ethers, alkyl halides & aliphatic amines present in the crude extracts alone and not by DMSO or pH of the extract which was neutralized during the experiment. Ernawati & Hasmila (2016) reported that the secondary metabolites present in the leaf extract of *R. mucronata* were flavonoids and steroid compounds. The presence of phenol and flavonoid contents of the *R. mucronata* has been proved for its antioxidant properties (Kaur *et al.*, 2019). Treatment of the crude extract showed a weak level of phospholipase against *V. harveyi*. It also revealed strong hydrophobic activity on the 30th day in the control experiment indicating, therefore, that the extract definitely can be used to control *V. harveyi*. The *R. apiculata* extract treatment, showed a weak level of phospholipase produced from *V. harveyi* and showed moderate

Table 4. Effect of extracts of R. mucronata against cumulative percent mortality (CPM) decrease in *P. monodon* postlarvae caused by *V. harveyi.*

Variables	Days						
	0	5	10	15	20	25	30
CPM							
Control (PL with V. harveyi)	0	4.0 ± 0.2	11.1 ± 0.5	21.0 ± 0.9	46.1 ± 1.6	58.8 ± 2.0	66.5 ± 2.2
Treatment (PL with extract and *V. harveyi*)	0	3.0 ± 0.2	12.0 ± 0.3	18.6 ± 1.4	34.6 ±1.2	46.9 ±1.7	50.8 ±2.1
Control (PL with extract)	0	2.9 ± 0.1	5.8 ±0.4	10.9 ± 0.2	11.0 ± 0.2	12.51±0.4	16.9 ±2.0
Control (PL alone)	0	4.0 ±0.1	8.1 ±0.2	15.5 ±0.4	27.7 ±0.3	30.3 ±1.5	35.1 ±1.0
Treatment tubs (CFU/mL)							
Total plate count	4.3×10^4	3.8×10^4	5.9×10^3	4.5×10^3	2.2×10^3	1.0×10^3	1.2×10^3
V. harveyi count	5.2×10^4	6.2×10^3	5.7×10^3	3.0×10^3	1.7×10^3	2.0×10^3	3.2×10^3
Total plate count	6.9×10^4	2.0×10^4	8.0×10^3	4.0×10^3	1.5×10^3	1.3×10^3	7.2×10^3
V. harveyi count	9.0×10^5	1.6×10^4	8.9×10^3	5.7×10^3	1.2×10^3	1.1×10^3	1.0×10^3
Average body weight of PL (mg)							
Treatment tubs	18.5 ± 2	61 ± 2	113 ± 2	165 ± 2	215 ± 2	262 ± 2	268 ± 3
Control tubs	18.0 ±3	57.6 ± 2	120 ± 2	162.8 ± 3	212 ± 3	259.2 ± 6	291.9 ±21
Water quality variables (Treatment and Control)							
Temperature (°C)	28 ± 1.0	28 ± 1.0	28 ± 1.0	29 ± 1.0	28 ± 1.0	29 ± 1.0	28 ± 1.0
Salinity (PSU)	18 ± 0.5	18 ± 0.5	18 ± 0.5	18 ± 0.5	19 ± 0.5	19 ± 0.5	19 ± 0.5
pH in control tubs	8.2 ± 0.2	8.3 ± 0.2	8.1 ± 0.2	8.3 ± 0.2	8.2 ± 0.2	8.1 ± 0.2	8.3 ± 0.2
pH in treatment tubs	8.3 ± 0.2	8.3 ± 0.2	8.2 ± 0.2	8.3 ± 0.2	8.2 ± 0.2	8.3 ± 0.2	8.3 ± 0.2

Values are the average of three determinations with a standard deviation (SD). PL: postlarvae. PSU: practical salinity unit (1 PSU = 1g/kg).

to weak hydrophobic activities (Kannappan *et al.*, 2018). The methanol extract of *R. apiculata* and *R. mucronata* (1.0 mg/mL) showed a reduction of the virulence factors like protease, pyocyanin pigments, and biofilm produced by *Pseudomonas aeruginosa* (Musthafa *et al.*, 2013). Quebrachitol, a bioactive compound extracted from the *R. mucronata* inhibited biofilm and virulence production in *Staphylococcus epidermidis* by impairment of initial attachment and intercellular adhesion (Karuppiah & Thirunanasambandham, 2020). The extract of *R. mucronata* showed a maximum decrease in bioluminescence production on the 15th day and the minimum decrease was on the 25th day. The production of intracellular luciferase also decreased on the 30th day. The methanol extracts of *R. apiculata* and *R. mucronata* showed significant inhibition against quorum-sensing dependent virulence factors such as protease, elastase, and production of pyocyanin and biofilm in P. aeruginosa PAO1 (Annapoorani *et al.*, 2013).

We found numerous significant biochemical compounds in *R. mucronata* (Table 3). In our study, the main phytoconstituents detected from *R. mucronata* were tetramethyl-6,7,8,8a-tetrahydro-5H-naphthalene-1-one, squalene, α-amyrin, and β-amyrin. In particular, the tetramethyl compounds have been reported to exhibit anticancer and antioxidant activity (Mohammed *et al.*, 2016). Also, bioactive components such as alkaloid, tannin, saponin and flavonoid extracted from the ripe fruit of *R. mucronata* were found to lower the blood glucose level in rats (Hardoko *et al.*, 2015). The squalene detected from the *R. mucronata* extract is an organic compound normally obtained from shark liver oil for commercial purposes. Adhikari *et al.* (2018) reported that *R. mucronata* leaves contained magnesium and squalene contents. *R. mucronata*-based squalene also may be used in the formulation of a wide variety of cosmetic products. Palaniyandi *et al.* (2018) found *in-vitro* anti gastric cancer activity of squalene, isolated from *R. mucronata* leaves against AGS cell line.

During the *P. monodon* larviculture, the mortality was reduced significantly when the extract was used to a considerable level. The CPM caused by *V. harveyi* decreased in postlarvae, (12-16% from 20-30th days by treating with the extract). Saptiani *et al.* (2019) reported that the ethanol extract (1500 ppm) of *R. mucronata* leaves inhibited *V. harveyi* and protected the tiger shrimp from infection with an improved survival rate and this treatment was akin to one using antibiotics. Dietary administration of crude extract of *R. mucronata* was found to enhance the growth response and innate immunity of clownfish (Dhayanithi *et al.*, 2020). Fermented mangrove propagules of *R. mucronata* have been used in fish feed, causing no negative effects on the growth of Nile tilapia fish fry (Andriani *et al.*, 2018). The crude extract of *R. apiculata* at 200 µg/mL, when tested against *V. harveyi* during larviculture of *P. monodon*, reduced mortality by 10.6% (Kannappan *et al.*, 2018). Nurhidayah & Atmomarsono (2020) reported on the potential of *R. mucronata* against disease-causing *V. harveyi* in tiger shrimp, with a MIC ranging between 1.0 and 10,000 mg/L. Similarly, the use of bark extract of *R. mucronata* extract (64.0 ppm) also resulted in curing *V. harveyi* infection with an enhanced survival rate (76.66%) in Nile Tilapia fish (Mulyani *et al.*, 2020). Also, the fruit extract of *R. stylosa* showed anti-*vibrio* activities as the cause of *Vibriosis* in mangrove crab larvae (*Scylla serrata*) (Burhanuddin *et al.*, 2019).

The growth of postlarvae increased when treated with the *R. mucronata* extract treatment coupled with the decreased total heterotrophic bacterial load. Even, *R. apiculata* plantation improved the water quality, growth, and health of mud crab (*Scylla paramamosain*) during the grow-out system, and promoted gut microbiota (Dai *et al.*, 2020). *R. mucronata* has been used in traditional medicine as an antiseptic, antibacterial, and anti-inflammatory agent (Suganthy & Devi, 2016). The values observed from the bio-assay of *R. mucronata* extract against the *V. harveyi* during *P. monodon* larviculture (p<0.05). Our study revealed significant differences between the extract-treated *V. harveyi* infection and control. Also exposed the anti-*vibrio* and anti-virulence potential of *R. mucronata* extract against bioluminescent *V. harveyi* and forms the baseline information for further research on other mangrove species.

In summary, our results reveal that the extracts of *R. mucronata* hold potent phytoconstituents which inhibit the growth and modulate the virulence factors produced by *V. harveyi*. Further, the extract was found to control the mortality caused by *V. harveyi* in the *P. monodon* larviculture and could be used as an alternative agent against aquaculture bacterial pathogens. Moreover, the purification of crude extracts of *R. mucronata* will provide huge opportunities on the detection of many bioactive compounds that could be utilized for making eco-friendly therapeutics. The chemical constituents inherent in *R. mucronata* were also presented. With the succeeding research on this plant, it is apparent that there are still unexplored new impending compounds obtainable from this plant. The cost of production of extract would be lower as compared to molecular grade chemicals used in animal experiments. The application of such bio-products would reduce the undesirable effects caused by the use of chemical preservatives in aquaculture with reduced production cost and also be eco-friendly. Furthermore, we intend to assess the effect of *R. mucronata* extract on the equilibrium between the growth and survival among the *P. monodon* postlarvae against *V. harveyi* in follow-up studies.

References

Abraham TJ, Palaniappan R, 2004. Distribution of luminous bacteria in semi-intensive *Penaeid* shrimp hatcheries of Tamil Nadu, India. Aquaculture 232: 81-90. https://doi.org/10.1016/S0044-8486(03)00485-X

Adhikari A, Ray M, Das AK, Sur TK, 2016. Antidiabetic and antioxidant activity of *Rhizophora mucronata* leaves (Indian Sundarnab mangrove) an *in-vitro* and in-vivo study. AYU 37: 76-81. https://doi.org/10.4103/ayu.AYU_182_15

Adhikari A, Ray M, Sur TK, Biswas S, Roy RK, Hazra AK, Das AK, 2018. Anti-diabetic activity of *Rhizophora mucronata* leaves in streptozotocin-nicotinamide induced animal model. J Middle East North Afr Sci 4 (8): 1-7.

Aljaghthmi OH, Heba HM, Abu Zeid IM. 2017. Anti-hyperglycemic properties of mangrove plants (*Rhizophora mucronata* and *Avicennia marina*): an overview. Adv Biol Res 11 (4): 161-170.

Amir Neori, 2011. Green water microalgae, the leading sector in world aquaculture. J Appl Phycol 23: 143-149. https://doi.org/10.1007/s10811-010-9531-9

Ananda Raja R, Sridhar R, Balachandran C, Palanisammi A, Ramesh S, Nagarajan K, 2017. Pathogenicity profile of *Vibrio parahaemolyticus* in farmed Pacific white shrimp, *P. vannamei*. Fish Shellfish Immunol 67: 368-381. https://doi.org/10.1016/j.fsi.2017.06.020

Andriani Y, Nurfalah F, Yustiati A, Iskandar, Zidni I, 2018. Utilization of fermented propagules (*Rhizophora mucronata*) as feeding material for Nile Tilapia. (*Oreochromis niloticus*) World Sci news 111: 74-86.

Annapoorani A, Kalpana B, Musthafa KS, Karutha Pandian S, Veera Ravi A, 2013. The antipathogenic potential of Rhizophora spp. against the quorum sensing mediated virulence factors production in drug-resistant *Pseudomonas aeruginosa*. Phytomedicine 20 (11): 956-963. https://doi.org/10.1016/j.phymed.2013.04.011

Arulkumar A, Sampath Kumar K, Paramasivam S, 2020. Antibacterial and *in-vitro* antioxidant potential of Indian mangroves. Biocatal Agr Biotechnol 23:1-10. https://doi.org/10.1016/j.bcab.2019.101491

Arunprabu S, Dinesh P, Ramanathan T, 2016. Antimicrobial activity of crude methanolic extracts of *Rhizophora mucronata.* Int J Sci Invent Today 5 (6): 520-527.

Baskaran R, Mohan PM, 2012. *In vitro* antibacterial activity of leaf extracts of *Rhizophora mucronata* against multidrug-resistant *vibrio* species isolated from marine waters Lobster's larvae hatcheries. Ind J Geo Mar Sci 41 (3): 218-222.

Beshiru A, Okareh OT, Okoh OA, Igbinosa EO, 2020. Detection of antibiotic resistance and virulence genes of *vibrio* strains isolated from ready-to-eat- shrimps in Delta and Edo states of Nigeria. J Appl Microbiol 129: 17-36. https://doi.org/10.1111/jam.14590

Biswas G, Ananda Raja R, De D, Sundaray JK, Ghoshal TK, Shyne Anand PS, *et al.*, 2012. Evaluation of production and economic returns of two brackishwater polyculture systems in tide-fed ponds. J Appl Ichthyol 28: 116-122. https://doi.org/10.1111/j.1439-0426.2011.01909.x

Burhanuddin A, Saru A, Rantetontok E, Nurhaida, Zainuddin EN, 2019. Antibacterial activity of Rhizophora stylosa and *Avicennia marina* of mangrove fruit extraction on *Vibriosis* of Mangrove Crab larvae (*Scylla serrata* Forsskal). Int J Env Agr Biotechnol 4 (4): 1242-1248. https://doi.org/10.22161/ijeab.4452

Chitra J, Syed Ali M, Anuradha V, 2019. Identification of bioactive compounds in the crude bark extract of *Rhizophora mucronata* by GC-MS analysis and HPLC fingerprinting of the crude and its fractions. Univ Rev 8 (6): 29-39.

Dai W, Xiong J, Zheng H, Ni S, Ye Y, Wang C, 2020. Effect of Rhizophora apiculata plantation for improving water quality, growth, and health of mud crab. Appl Microbiol Biotechnol 104: 6813-6824. https://doi.org/10.1007/s00253-020-10716-7

Das BK, Pradhan J, Pattnaik P, Samantaray BR, Samal SK, 2005. Production of antibacterials from the freshwater alga *Euglena virdis* (Ehren). World J Microbiol Biotechnol 21: 45-50. https://doi.org/10.1007/s11274-004-1555-3

Defoirdt T, Halet D, Vervaeren H, Boon N, Vande Wiele T, Sorgeloos P, *et al.*, 2007. The bacterial storage compound poly-β-hydroxybutyrate protects *Artemia ranciscana* from pathogenic *V.campbellii.* Environ Microbiol 9: 445-452. https://doi.org/10.1111/j.1462-2920.2006.01161.x

Defoirdt T, Boon N, Sorgrloos P, Veratraete W, Bossier P, 2008. Quorum sensing and quorum quenching in *Vibrio harveyi*: lessons learned from in vivo work. The ISME journal 2: 19-26. https://doi.org/10.1038/ismej.2007.92

Dhayanithi NB, Ajithkumat TT, Arockiaraj J, Balasundaram C, Ramasamy H, 2015. Immune protection by Rhizophora apiculata in clownfish against *Vibrio alginolyticus.* Aquaculture 446: 1-6. https://doi.org/10.1016/j.aquaculture.2015.04.013

Dhayanithi NB, Kumar TTA, Balasubramoniam C, Devi G, Ramasamy H, 2020. Immuno antioxidant defence of partially purified *Rhizophora mucronata* in clownfish (*Amphiprion sebae*) against *Vibrio alginolyticus.* J Cell Mol Biol 4 (9): 1-10.

D'Souza L, Devi P, Shridhar DM, Naik CG, 2008. Use of Fourier Transform Infrared (FTIR) spectroscopy to study cadmium-induced changes in *Padina tetrastromatica* (Hauck). Anal Chem Insights 3: 135-143. https://doi.org/10.4137/117739010800300001

Ernawati, Hasmila I, 2016. Phytochemical and antibacterial activity test of secondary metabolite compound in *Rhizophora mucronata* methanol leaves extracts. Proc 3rd Int Conf on Research, Implementation and Education of Mathematics and Science, Yogyakarta, 16-17th May, pp:115-119.

Ganesh Kumar A, Arun G, Vinod Kumar S, Raja Ram R, 2019. Bioaccumulation and translocation efficiency of heavy metals by *Rhizophora mucronata* from tropical

mangrove ecosystem, Southeast coast of India. Echohydr Hydrobiol 19: 66-74. https://doi.org/10.1016/j.ecohyd.2018.10.006

Hardoko ES, Suprayitno E, Puspitasari YE, Amalia R, 2015. Study of ripe *Rhizophora mucronata* fruit flour as functional food for antidiabetic. Int Food Res J 22 (3): 953-959. http://www.ifrj.upm.edu.my/22%20(03)%202015/(11).pdf

Harris L, Owens L, Smith S, 1996. A selective and differential medium for *V. harveyi*. Appl Environ Microbiol 62: 3548-3550. https://doi.org/10.1128/aem.62.9.3548-3550.1996

Igbinosa IH, Beshiru A, Igninosa EO, 2017. Antibiotic resistance profile of *Pseudomonas aeruginosa* isolated from aquaculture and abattoir environments in urban communities. As Pac J Trop Dis 7: 930-935.

Islam MA, Alam MM, Choudhury ME, Kobayashi N, Ahmed MU, 2008. Determination of minimum inhibitory concentration of cloxacillin for selected isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) with their antibiogram. Bangl J Vet Med 6 (1): 121-126. https://doi.org/10.3329/bjvm.v6i1.1350

Kannappan S, Sivakumar K, Seti S, 2018. Protective effect of mangrove (Rhizophora apiculata) leaves extract in shrimp (*Penaeus monodon*) larvae against bio-luminescent disease-causing *Vibrio harveyi* bacteria. Span J Agric Res 16 (1): e0501. https://doi.org/10.5424/sjar/2018161-11675

Kannappan S, Sivakumar K, Patil PK, 2013. Effect of garlic extract on the luciferase, bio-luminescence, virulence factors produced by *Vibrio harveyi* with a challenge during *Penaeus monodon* larviculture. Afr J Microbiol Res 7 (18): 1766-1779. https://doi.org/10.5897/AJMR12.950

Karuppiah V, Thirunanasambandham R, 2020. Quebrachitol from *Rhizophora mucronata* inhibits biofilm formation and virulence production in *Staphylococcus epidermidis* by impairment of initial attachment and intercellular adhesion. Arch Microbiol 202: 1327-1340. https://doi.org/10.1007/s00203-020-01844-9

Kaur S, Yacoob SAM, Venktraman A, Nagarajan Y, Vasudevan S, Punniyamoorthy B, 2019. Proximate composition and *in vitro* antioxidant properties of *Rhizophora mucronata* plant part extract. As J Green Chem 3 (3): 345-352.

Kesavaraju PV, Sreeramulu K, 2017. Antimicrobial activities of marine resources against the fish pathogens isolated from *Mugil cephalus*. Int J Fish Aquatic Sci 7 (1): 1-13.

Manilal A, Sujith S, Selvin J, Kiron CS, Shakir C, Lipton AP, 2010. Antimicrobial potential of marine organisms collected from the South West coast of India against multi resistant human and shrimp pathogens. Scientia Marina 74 (2): 287-296. https://doi.org/10.3989/scimar.2010.74n2287

Manilal A, Merdekios B, Idhayadhulla A, Muthukumar C, 2015. An *in-vitro* antagonistic efficacy validation of R.mucronata. As Pac J Trop Dis 5 (1): 28-32. https://doi.org/10.1016/S2222-1808(14)60622-8

Mohammed CJ, Omran AS, Hussein HM, 2016. Antibacterial and phytochemical analysis of Piper nigrum using GC-MS, mass spectrum, and FT-IR spectroscopy. Int J Pharmacog Phytochem Res 8 (6): 977-996.

Mulyani Y, Haetami K, Baeha LK, Arsad S, Prasetiya FS, 2020. In vivo test of *Rhizophora mucronata* mangrove extract from Pangandaran Coast towards *Nile Tilapia, Oreochromis niloticus* infected by *Vibrio harveyi*. J Aquacult Fish Health 9 (2): 131-142. https://doi.org/10.20473/jafh.v9i2.16211

Musharraf SG, Ahmed MA, Zehra N, Kabir N, Choudhary MI, Rahman AU, 2012. Bio-diesel production from microalga isolates of southern Pakistan and quantification of FAME's by GC-MS/MS analysis. Chem Cent J 6 (1): 1-10. https://doi.org/10.1186/1752-153X-6-149

Musthafa KS, Sahu SK, Ravi AV, Kathiresan K, 2013. Anti-quorum sensing potential of the mangrove Rhizophora annamalayana. World J Microbiol Biotechnol 29 (10): 1851-1858. https://doi.org/10.1007/s11274-013-1347-8

Nurhidayah M, Atmomarsono M, 2020. Potency of mangrove *Rhizophora mucronata* as a bactericide for *vibrio* causing tiger shrimp disease. 3rd Int Symp Mar Fish (ISMF), IOP Conf Series Earth Environ Sci 564: 012031. https://doi.org/10.1088/1755-1315/564/1/012031

Palaniyandi T, Sivaji A, Thiruganasambandam R, Natarajan S, Hari R, 2018. *In vitro* anti gastric cancer activity of squalene, a triterpenoid compound isolated from *Rhizophora mucronata* mangrove plant leaves against AGS cell line. Phcog Mag 14: S369-76. https://doi.org/10.4103/pm.pm_577_17

Saptiani G, Asikin AN, Ardhani F, Hardi EH, 2019. The potential of *Rhizophora mucronata* extracts to protect tiger prawn from pathogenic infections. I Malaku Int Conf Mar Sci Technol, IOP Conf series Earth Environ Sci 339: 012049. https://doi.org/10.1088/1755-1315/339/1/012049

Sivakumar K, Kannappan S, 2013. Inhibitory effect of marine algae collected from the East and West coast of India against luciferase and luminescence producing *Vibrio harveyi*. Afr J Biotechnol 12 (22): 3493-3502.

Soto-Rodriguez SA, Gomez-Gil B, Lozano R, del Rio-Rodriguez R, Dieguez AL, Romalde JL, 2012. Virulence of *V. harveyi* responsible for the "Bright-red" syndrome in the Pacific white shrimp Litopenaeus vannamei. J Invertebr Pathol 109 (3): 307-317. https://doi.org/10.1016/j.jip.2012.01.006

Suganthy N, Devi KP, 2016. Protective effect of catechin-rich extract of *Rhizophora mucronata* against amyloid-induced toxicity in PC12 cells. J Appl Biomed 14 (2): 137-146. https://doi.org/10.1016/j.jab.2015.10.003

Sur T, Hazra A, Bhattacharyya D, Hazra A, 2015. Anti-radical and antidiabetic properties of standardized extract of Sunderban mangrove *Rhizophora mucronata*. Pharmacogn Mag 11 (42): 389-394. https://doi.org/10.4103/0973-1296.153094

Traifalgar RF, Serrano AE, Corre V, Kira H, Tung HT, Fady RM, *et al.*, 2009. Evaluation of dietary fucoidan supplementation effects on growth performance and *Vibriosis* resistance of *Peneaus monodon* postlarvae. Aquacult Sci 57: 167-174.