World Applied Sciences Journal 25 (2): 333-340, 2013
ISSN 1818-4952

DOI: 10.5829/idosi.wasj.2013.25.02.688

# Antibacterial Properties of Selected Mangrove Plants Against *Vibrio* Species and its Cytotoxicity Against *Artemia salina*

[1,2]A.A. Shamsuddin, [3]M. Najiah, [1]A. Suvik, [2]M.N. Azariyah,
[4,5]B.Y. Kamaruzzaman, [1]A.W. Effendy and [4,5]B. Akbar John

[1]Institute of Marine Biotechnology,
[2]Faculty of Maritime Studies and Marine Science,
[3]Faculty of Agrotechnology and Food Science,
Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Malaysia
[4]Institute of Oceanography and Maritime studies (INOCEM),
[5]Kulliyyah of Science, International Islamic University Malaysia,
Jalan Sultan Ahmad Shah, 25200 Kuantan, Pahang, Malaysia

**Submitted:** Jul 6, 2013; **Accepted:** Aug 15, 2013; **Published:** Oct 1, 2013

**Abstract:** Emerging antibiotic resistance among the pathogenic microorganism has triggered the discovery of various antimicrobial compounds from marine source. Secondary metabolites from plants especially mangroves is traditionally being used widely as antimicrobials. Hence, present study was aimed to determine the antibacterial and cytotoxic properties of the selected mangrove plant leaves (*Bruguiera cyclindrica, Sonneratia caseolaris, Luminitzera racemosa, Rhizophora apiculata, Avicennia alba, Acrostichum aureum, Nypa fruticans, Pandanus odoratissimus, Hibiscus tiliaceous* and *Derris trifoliate*) against aquaculture pathogenic strains of genus. Methanol and aqueous extracts of leaves samples were prepared. The antibacterial activity was determined by using disc diffusion method against six pathogenic Gram-negative *Vibrio* species, such as *Vibrio alginolyticus* and *V. parahaemolyticus* (isolated from *Scylla serrata,* mangrove crab), *V. alginolyticus* and *V. parahaemolyticus* (from Giant prawn), *V. alginolyticus* (from Tiger prawn) and also *V. parahaemolyticus* (from Reference strain ATCC 17802). Methanolic extracts of *S. caseolaris* produced significant inhibition zone against all the pathogenic *Vibrio* spp. except for *V. parahaemolyticus* (from Giant prawn). Minimum inhibitory concentration test (MIC) and cytotoxicity ($LC_{50}$) test using *Artemia salina* as a test animal in sterile ELISA (Enzyme-Linked Immunosorbent Assay) microplates showed the significant antimicrobial properties of methanolic extract of *S. caesolaris*. Overall, present study proved the active antimicrobial compounds in methanolic extract of *S. caesolaris* which could be used against *Vibrio* pathogens in aquaculture.

**Key words:** Antibacterial properties · *Vibrio* sp. · Solvent extract · Mangrove plant · Cytotoxicity · *Artemia salina*

## INTRODUCTION

Recently, some 12 species of the *Vibrio* inhabiting in marine and estuary environments has been reported to be the disease causing pathogens in humans [1]. Human pathogenic *Vibrio* species were also detected in temperate waters (especially during the summer), but at lower frequencies than in tropical waters. Selectively some species of *Vibrio* such as *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus* are commonly associated with disease problems which related to an ingestion or other routes of contaminated waters [2]. The *V. vulnificus* has been associated with wound infection and septicaemia, meanwhile the *V. cholera* and *V. parahaemolyticus* are mainly cause gastro-intestinal symptoms. In Japan, *V. parahaemolyticus* has been

**Corresponding Author:** B.Y. Kamaruzzaman, Kulliyyah of Science, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, 25200 Kuantan, Pahang, Malaysia.
Ph: 0129085734

reported to be the most common causative agent of food poisoning in fisheries industry [3]. These *Vibrio* species are commonly found on shellfishes and all varieties of fishes that taken from marine, aquaculture industry [4]. These bacterial are also compatible with a marine or brackish water environment and could adjust well to the broad range of salinities [5]. To overcome the infection and contamination problems in aquaculture and food industry, antimicrobial agents such as antibiotics has been widely used as solution and precaution. However, several studies have assessed the impact of antimicrobial agents in aquaculture towards the bacteria in the local environment. In Malaysia, rapidly growing aquaculture industry contributes about 1.275 billion Malaysian ringgits in a year [6]. As antibiotics are increasingly used and misused in the industry, the bacterial strains become resistant to antibiotics rapidly. For example, *Aeromonas salmonicida* was resistant towards specific antibiotic just shortly after antibiotic have been introduced into aquaculture [6]. To delay this phenomenon, several measures have been put in place including the establishment of maximum residue levels in fish destined for consumption and the requirement of elaborate approval procedures as well as guidelines for use of new antibiotics [7]. This reflects the concern among the scientific and health communities that overuse of antibiotics could lead to a pre-antibiotic era all over again. The increased cases of antibiotic resistant among pathogenic bacteria has encourage scientist to find new drugs against these pathogenic bacteria which supported by increasing number of compound with antibacterial activity extracted from botanical and animals source [8]. Hence, extraction of antibacterial activity of medicinal plants especially from different parts of the mangrove plants is very important since vast number of medicinal plants have been used for centuries as remedies for human diseases [9].

Medicinal properties of mangrove plants have been discovered centuries ago, for example, a physician in Cali, Colombia, reported to cure throat cancer, with gargles of mangrove bark meanwhile, bark of red mangrove trees have been used in folk remedy for a wide array of diseases which associated with the common cold such as nasal congestion, bronchial congestion, runny nose and many more [10, 11]. Mangroves have been found to contain high antioxidant activity and antiradical scavenging activity, inhibited viruses and as antibacterial and antifungal [11, 12]. Realizing the potential of these mangrove plants, present study was conducted to determine the antibacterial activity of leaves of selected mangrove plant leaves (*Bruguiera cyclindrica, Sonneratia caseolaris, Luminitzera racemosa, Rhizophora apiculata, Avicennia alba, Acrostichum aureum, Nypa fruticans, Pandanus odoratissimus, Hibiscus tiliaceous* and *Derris trifoliate*) against *Vibrio* species isolated from some marine organisms.

## MATERIALS AND METHODS

A total of 10 mangrove species (*Bruguiera cyclindrica, Sonneratia caseolaris, Lumnitzera racemosa, Rhizophora apiculata, Avicennia alba, Acrostichum aureum, Nypa fruticans, Pandanus odoratussimus, Hibiscus tiliaceous* and *Derris trifoliata*) leaves were collected from Universiti Malaysia Terengganu estuarial area. The leaves were washed with running tap water followed by distilled water sevelal time to remove the debris and oven dried at 60°C for 5 days.

**Preparation of Plant Extracts:** Methanol and aqueous extracts of dried leaf samples were prepared using 80 % methanol and sterile water (450 ml) respectively for three days and then filtered. 15g of powdered leaf were soaked in methanol and distilled water for 3 days and filtered using Whatman No1 paper and evaporated to a thick residue at 40°C using rotary evaporator (methanol extract) and lyophilized (distilled water extract). The dry-crude extracts were irradiated with ultraviolet light for sterilization. All extracts were dry-stored in sterile eppendorf at 4°C until further used.

**Preparation of Discs**

**Discs of Crude Extracts:** Sterile filter paper discs were placed in a sterile plate contained Mueller Hinton agar (MHA). 10 µl of sample solution was transferred on the discs aseptically. Standard antibiotic such as chloramphenicol and tetracycline discs were used as positive control and solvents (methanol and sterile water) were used as negative control.

**Tested Bacterial Strains:** The bacterial strains were procured from Fish Disease Laboratory of Universiti Malaysia Terengganu. A total of six *Vibrio parahaemolyticus* and *V. alginolyticus* isolated and identified from different hosts were used. They were *V. alginolyticus* and *V. parahaemolyticus* (from Giant Prawn), *V. alginolyticus* (PCY 24) and *V. parahaemolyticus* (PC 29) (from mangrove crab, *Scylla serrata*), *V. alginolyticus* (from Tiger prawn) and *V. parahaemolyticus* (from reference strain ATCC No 17802).

**Determination of Antibacterial Activities:** Disc diffusion method was carried out to determine the antibacterial activity of the crude extracts following Bauer *et al*. (1966) method [13]. 0.2 grams of crude extracts were added with 1000 µl of 10 % methanol. The discs were prepared by impregnating 10 µl of diluted extracts and then they were placed onto the MHA which was previously swabbed with the suspension of bacteria. Standard disc of chloramphenicol and tetracycline were used, as positive control and disc containing methanol or sterile water acted as negative control. Then the plates were incubated for 24 hours at 30°C. The antibacterial activities were measured based on the inhibition diameter zone around the disc.

**Determination of Minimum Inhibitory Concentration (MIC):** The minimum inhibitory concentration (MIC) test was carried out by using ELISA plate assay range from 100 mg/ml to 0.1 mg/ml [14]. The MIC's value for a drug was expressed as the lowest concentration that inhibits the bacterial growth (Europian Society of Clinical Microbiology and Infectious Disease 2003). All wells were filled with 95ul of Trypticase Soy Broth (TSB) and diluted crude extracts by two-fold dilution. Then, 5 µl of bacteria solution tested were transferred into each well containing TSB and also extracts. The growth of bacteria's optimal density (OD) and the suspension of the OD were determined before and after 48 hour of incubation by using ELISA reader at 540 nm. All the tests were performed in triplicate for better statistical prediction.

**Determination of Toxicity, $LC_{50}$ activity**

**Hatching of Artemia Eggs:** A total of 15 mg of dried brine shrimp eggs were submerged into a beaker containing 500 ml seawater and were supplied with oxygen throughout the night. The eggs hatched later were incubated in darkness for 24 hours at 25°C.

**Larvae Collection and Concentration:** Phototropic Nauplii stages attracted with the light source (at the top of the beaker) were transferred into sterile polystyrene petri dish contained 5 ml fresh artificial supplemented seawater to easily transfer the nauplii into the ELISA microplates well.

**Bioassay:** The toxicity assay was performed by using ELISA microplates method [15, 16]. Each well was added with 90 µl of seawater into two-fold serial dilution. Then, 10 µl of seawater which contain 10 ± nauplii was added to each well. After 24 hours, the ELISA microplates were viewed under dissecting microscope to observe the minimal concentration of the extracts that allows the nauplii to live. Nauplii that remained immobile were considered dead. Control test were performed in 100 µl seawater containing 10 ± nauplii without extracts.

**Statistical Analysis:** All data were analyzed by using One-Sample Kolmogorov-Smirnov Goodness-of-Fit test to confirm normal distribution of *Sonneratia caseolaris* extracts against *Artemia salina* mortality rate to check the doas response relationship and Probit analysis was done to determine the relative toxicity of chemicals to living organisms. Data were analysed using SPSS 17v.

## RESULTS

**Crude Extraction:** *Nypa fruticans* and *Rhizophora apiculata* showed the highest weight and percentage in terms of yield extraction via both extraction (methanol and distilled water) with 2.4 and 2.7 mg/g respectively. The percentage of yield extractions was determined by the sample: solvent ratio 1:2. Most of the colors obtained from the crude extracts were of dark brown, light brown and also dark green (Table 1).

**Diameter of Inhibition Zone of Impregnated Disc:** The plant extracts showed positive results against the gram negative *Vibrio* spp. bacteria except for *L. racemosa* and *P.odoratissimus* which showed no inhibition activity against all six isolates (Table 2). Among all plants tested, only *S. caseolaris* via methanol extraction exhibited better and stronger antibacterial activities against *V. alginolyticus* and *V. parahaemolyticus* isolates with inhibition zones diameter of 18.3 mm compared to other plants while *B. cyclindrica* exhibited the strongest antibacterial activity for aqueous extraction with diameter of 11.7 mm followed by *R. apiculata* (11.3 mm). *Acrostichum aureum, N. fruticans, H. tiliaceus* and *D. trifoliata* showed inhibition activity against *V. parahaemolyticus* (*Scylla serrata*), *V. alginolyticus* (Giant Prawn), *V. alginolyticus* (*Scylla serrata*) and *V. alginolyticus* (Giant Prawn) respectively. Meanwhile, *A. alba* also showed active inhibition activity against five *Vibrio* spp. except for reference strain *V. parahaemolyticus*.

**Minimum Inhibitory Concentration (MIC) of S. Caseolaris:** Among the ten mangrove plants tested, *S. caseolaris* showed the greater inhibition activity against all pathogenic strains and thus it was further tested for MIC test (Table 3). Two types of extraction;

Table 1: Dry weight of crude leaves extract and percentage of yield extraction and colors of crude extract obtained.

| | | Weight of crude leaves extracts (mg/g) & % of yield extraction | | | | Colors of crude extracts | |
|---|---|---|---|---|---|---|---|
| Species name | Dry weight (g) | Methanol | % | Aqueous | % | Methanol | Aqueous |
| *Bruguiera cyclindrica* | 68.3 | 2.1 | 3.1 | 2.06 | 3.0 | Dark brown | Light brown |
| *Acrostichum aureum* | 21.5 | 0.9 | 3.9 | 0.8 | 3.8 | Dark brown | Light brown |
| *Avicennia alba* | 53.4 | 2.0 | 3.8 | 2.0 | 3.8 | Light brown | Dark brown |
| *Lumnitzera racemosa* | 50.1 | 1.6 | 3.2 | 1.5 | 2.9 | Dark brown | Light brown |
| *Sonneratia caseolaris* | 53.3 | 2.4 | 4.5 | 2.3 | 4.2 | Light brown | Dark brown |
| *Pandanus odoratissimus* | 55.5 | 1.7 | 3.0 | 1.5 | 2.8 | Dark green | Dark brown |
| *Nypa fruticans* | 68.2 | 2.4 | 3.5 | 2.3 | 3.4 | Dark green | Light brown |
| *Hibisicus tiliaceus* | 22 | 1.7 | 7.5 | 1.6 | 7.1 | Dark green | Dark green |
| *Rhizophora apiculata* | 50.6 | 2.6 | 5.0 | 2.2 | 4.4 | Dark green | Light brown |
| *Derris trifoliata* | 40.5 | 2.7 | 6.7 | 1.4 | 3.4 | Dark green | Dark brown |

Table 2: Diameter of Inhibition Zone (in millimeter) of the impregnated disc (10 µg/ml) against six *Vibrio* spp

| | | | | | V.A (S) Pcy 24 | | V.P (S) Pc 29 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *V.A GP* | | *V.P GP* | | | | | | V.A TP | | RF V.P | |
| Plants | A | M | A | M | A | M | A | M | A | M | A | M |
| *Bruguiera cyclindrica* | 11.7 | - | 2.7 | - | 6.7 | - | - | - | 2.7 | - | - | - |
| *Acrostichum aureum* | - | - | - | - | - | - | - | 1.3 | - | - | - | - |
| *Avicennia alba* | 8.7 | - | 9.3 | - | 9.3 | - | - | 5 | 9.3 | - | - | - |
| *Lumnitzera racemosa* | - | - | - | - | - | - | - | - | - | - | - | - |
| *Sonneratia caseolaris* | - | 16.7 | - | - | - | 17 | - | 12 | - | 11.3 | - | 18.3 |
| *Pandanus odoratissimus* | - | - | - | - | - | - | - | - | - | - | - | - |
| *Nypa fruticans* | - | 9.3 | - | - | - | - | - | - | - | - | - | - |
| *Hibiscus tiliaceus* | - | - | - | - | - | 5 | - | - | - | - | - | - |
| *Rhizophora apiculata* | - | 11.3 | - | - | - | - | - | 10 | - | - | - | - |
| *Derris trifoliata* | - | 1.3 | - | - | - | - | - | - | - | - | - | - |
| *Chloramphenicol,(C)* | - | 34.7 | 36.7 | 23.7 | 40 | 40.3 | 39 | 40.3 | - | 38.3 | - | 40 |
| *Tetracycline(TC)* | - | 24 | 6.7 | 2.7 | 8 | 9.3 | 26 | 29.7 | - | 25 | - | 26.3 |

*Note:* *A - Aqueous extraction, M- Methanol extraction; VA -*Vibrio alginolyticus*;VP- *Vibrio parahaemolyticus;* RF- Reference strain *ATCC 17802*. (S) - Mangrove crab (*Scylla serrata*); TP - Tiger prawn; GP - Giant Prawn

Table 3: Minimum concentration of *Sonneratia caseolaris* inhibiting the growth of bacteria

| | MIC, µg/ml | |
|---|---|---|
| Bacteria | Methanol extraction, µg/ml | Aqueous extraction, µg/ml |
| *RFVP* | 0.1 | 1.6 |
| *VPGP* | 0.1 | 1.6 |
| *VAGP* | 0.1 | 6.3 |
| *VATP* | 0.1 | 3.1 |
| *(S)PCY 24* | 0.2 | 1.6 |
| *(S)PC 29* | 0.1 | 1.6 |

**Notes*: MIC - Minimum inhibition zone; VA -*Vibrio alginolyticus* ; VP- *Vibrio parahaemolyticus*; RF- Reference strain *ATCC 17802;* GP - Giant Prawn; (S) - Mangrove crab (*Scylla serrata*); TP - Tiger prawn

methanol and aqueous extraction of *S. caseolaris* against six *Vibrio* spp. were tested to determine the minimum concentration of the extracts that inhibit the bacteria. Certain activity against this bacterium was shown by methanol extraction, which has the lowest MIC value (0.1 µg/ml) against all of the *Vibrio* spp. compared to aqueous extracts. Only methanol extraction of *S. caseolaris* against *PCY 24* stated different value (0.2 µg/ml). *RFVP, VPGP, PCY24, PC29* shared the same concentration 1.6 µg/ml for aqueous extraction for its MIC value while *VATP* and *VAGP* were recorded with the values of 3.1 µg/ml and 6.3 µg/ml respectively.

Table 4: One-Sample Kolmogorov-Smirnov Test of *Sonneratia caseolaris* aqueous and methanolic extracts against *Artemia salina* mortality rate

| One-Sample Kolmogorov-Smirnov Test (Aqueous extract) | | |
|---|---|---|
| | | Mortality |
| N | | 12 |
| Poisson Parameter[a] | Mean | 5.58 |
| Kolmogorov-Smirnov Z | | 1.530 |
| Asymp. Sig. (2-tailed) | | .018 |
| **One-Sample Kolmogorov-Smirnov Test (methanol extracts)** | | |
| | | Mortality |
| N | | 11 |
| Poisson Parameter[a] | Mean | 3.18 |
| Kolmogorov-Smirnov Z | | 1.973 |
| Asymp. Sig. (2-tailed) | | .001 |

*Note: a. Test distribution is Poisson. $H_0$: A sample is normally distributed; $H_1$: A sample is not normally distributed P >0.05.

Table 5: Probit analysis of *Sonneratia caseolaris* aqueous and methanolic extracts against *Artemia salina* mortality rate

| | | PROBIT ANALYSIS | | |
|---|---|---|---|---|
| Extract | | Chi-Square | df[a] | Sig. |
| Aqueous | Pearson Goodness-of-Fit Test | .513 | 8 | 1.000[b] |
| Methanol | | 10.654 | 9 | .300[b] |

a. Statistics based on individual cases differ from statistics based on aggregated cases.

.b. Since the significance level is greater than .150, no heterogeneity factor is used in the calculation of confidence limits

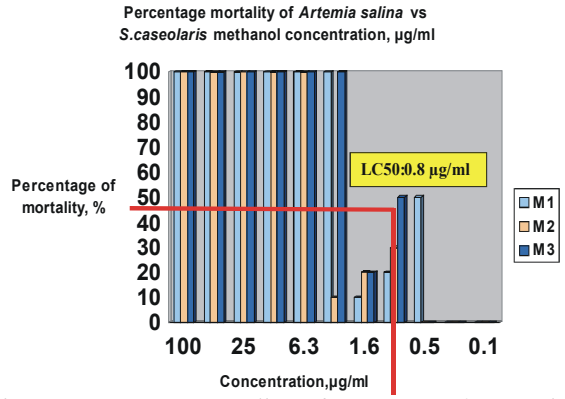


Fig. 1: Percentage mortality of *Artemia salina* against concentration of *Sonneratia caseolaris* after 12 hour exposure on different concentrations of the methanolic extracts. (M: Methanol)

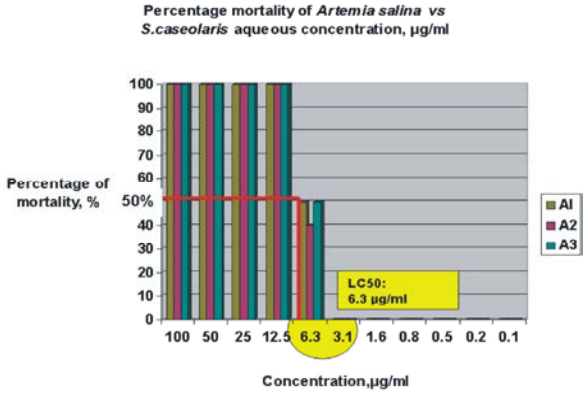


Fig. 2: Percentage mortality of *Artemia salina* against concentration of *Sonneratia caseolaris* after 12 hour exposure on different concentrations of the aqueous extracts. (A: aqueos)

**$LC_{50}$ of Sonneratia Caseolaris Methanolic Extracts Against Artemia Salina:** In mortality test, two fold serial dilution method was performed to get different concentrations from 100 µg/ml to 0.1 µg/ml in every well (11 well). The ELISA microtiter plate method showed positive correlation between percentage mortality rate and the concentration of the extract (Figure 1). The $LC_{50}$ for methanolic and aqueous extract of *S. caseolaris* was found to be at 0.8 µg/ml and 6.3 µg/ml respectively. Kolmogorov-Smirnov one-sample test for the aqueous and methnolic extract showed that the samples are not distributed normally (Table 4) which was further proved by Probit analysis that showed both extracts have significance level greater than 150 (Table 5). Due to this observation $LC_{50}$ for *Sonneratia caseolaris* extracts were determined by the simple percentage graph (Figure 1 and 2).

## DISCUSSION

In this study, the methanolic and aqueous extract of selected mangrove plants were tested against six different *Vibrio* species and their cytotoxic activity against

*Artemia salina* were checked. Results have shown that the methanolic and aqueous extracts of all the plant leaves were effective inhibition activity against tested bacterial isolates except *L. racemosa and P. odoratissimus* extracts. Martin (1995) reported that gram-negative bacteria are more resistant than gram-positive but in this study, most plants have antibacterial activities towards the *Vibrios* which indicates the inhibition of biochemical pathways that are involved in the biosynthesis of essential components of the bacterial cell [17, 18]. They also reported that the three main bacterial targets of antimicrobial agents are cell wall, protein and nucleic acid biosynthesis. The cell wall is also a porous barrier that is not selectively permeable, allowing passage of substances through the pores. Compared to gram-positive thick peptidoglycan layers, gram-negative bacteria peptidoglycan layer is thinner and surrounded by a lipopolysaccharide later as its outer membrane [19, 20]. Arthur *et al*.(1996) also stated that these structures confer lower permeability in comparison with gram-positive bacteria and prevent penetration of antimicrobials like glycopeptides that do not fit the size of the pores in the outer membrane, called porins [20]. The polypeptide contain in the plant tested could indicates that it is targeting the cell membrane of the gram-negative *Vibrios*. These drugs may have interact with the phospholipids of the cell membrane of gram-negative *Vibrio* species and increase its permeability and so it is easier for the drugs to inhibit the activities of the *Vibrio* species.

The activity of the plant extracts on different species of bacteria confers their broad spectrum nature while it is the opposite for plants that have effect on one strain bacteria only such as *Acrostichum aureum, Hibiscus tiliaceus* and *Derris trifoliata* that indicates their narrow spectrum activity. The difference rate of inhibition activities appear to be directly related to the qualitative and quantitative diversity of the compounds that are being accumulated by the plants as its accumulate secondary chemical, which it will reduce their nutritional quality or make them toxic to potential competitors or parasites [21]. The difference in potency may also due to the stage collection of the sample, sensitivity of the bacterial test strains and also method of extraction. These bacterial strains may also have some kind of resistance mechanisms e.g. enzymatic inactivation, target sites modification and decrease intracellular drug accumulation [22].

It was observed that the considerable reduction in antibacterial activity of plant extracts during the period of four months after extraction. It may be due to degradation or volatility of antibacterial compounds or they may have converted into non-antibacterial compounds. Particularly, extract of *Sonneratia caseolaris* methanol extraction has exhibited all antibacterial activities of *Vibrio* species tested respectively. *Sonneratia caseolaris* also exhibited the strongest antibacterial activity for methanol extraction with the greater inhibition zones (18.3 mm) compared to other plants while *Bruguiera cyclindrica* exhibited the strongest antibacterial activity for aqueous extraction with diameter reading of 11.7 mm followed by the others (Table 3).

*Sonneratia caseolaris* was further tested with minimum inhibition concentration (MIC) test with the value corresponding to methanol extraction, which has the lowest MIC value (0.1 µg/ml) against all of the *Vibrio* spp. compared to aqueous extraction reading. The secondary metabolites such as alkaloids, flavonoids and steroids in *S. caseolaris* may exert antibacterial activity against tested bacterial strains [22]. RFVP*, VPGP, PCY24, PC29* shares the same concentration 1.6 µg/ml for aqueous extraction for its MIC value while *VATP* was with 3.1 µg/ml and *VAGP* 6.3 µg/ml respectively. This result may be due to the possible presence of two flavonoids, luteolin and luteolin 7-O--glucoside and so it confers of the plant having antioxidant activity. Flavonoids glycosides, steroids and triterpernoids in *S. caseolaris* plant tissues were related to their biological activities [23]. It also has been reported having phenolic compounds including tannin that possess antioxidant activity [24]. It is possible that all these compounds may be responsible for the free radical scavenging activity of the plants from the mangrove forest. Some of the bacterial strains did not respond to some plant crude extracts such as *Pandanus odoratissimus* and *Lumnitzera racemosa* which might be due to the masking of antibacterial activity by the presence of some compounds or factors in the extract [24]. It is also stated that the variation of antibacterial activity of the extracts might be due to distribution of antimicrobial substances, which varied from fraction to fraction of the crude extract. Pattanaik *et al*. (2007) reported that the presence of tannin in *Bruguiera cyclindrica* might influenced in producing biggest inhibition zone compared to other tested plants in this study [25].

**Toxicity Test:** The brine shrimp (*Artemia salina* L.) mortality assay is considered a useful tool for preliminary assessment of toxicity [15]. These brine shrimps were exposed to various concentrations of the extract. The mortality was determined after 24 hours (mainly in

instar II/III; early life stages for newly hatch brine shrimp) of exposure where the larvae did not receive food. In any case, hatched brine shrimp nauplii can survive for up to 48 hr without food because they still feed on their yolk-sac [26]. The $LC_{50}$ for methanolic extract of *S. caseolaris* was found to be at 0.8 µg/ml and LC50 for aqueous extract of *S.caseolaris* was found to be at 6.3 µg/ respectively. In the mortality test, we could see the dependant relationship was observed wherein the percentage of *Artemia salina* mortality decreases as the concentration of the extract decrease. But the Kolmogorov-Smirnov one-sample test indicates that the aqueous and methanolic samples are not normally distributed ($p < 0.05$). Whereas Probit analysis assumes that the relationship between number responding (not percent response) and concentration is normally distributed but still, the analysis results showed that both sample are not normally distributed because the significance values are higher than 0.150; with aqueous and methanolic extracts significant value were 1.000 and 0.300. This may be due to the nauplii that were not fully achieved its maximum sensibility that usually reached after 48hr of exposure (the oldest age class tested) [27, 28]. At this maximum sensibility stage, the life cycle of the nauplii have reached second and third instars (the early life stages for newly hatch brine shrimp), which it has, exhibit greatest sensitivity towards the test compounds [29]. With these preliminary results, it is possible that *S.caseolaris* might contain substances that have cytotoxic activity. However, further work is needed to address this observation. It may also be possible that the non-active compound in the crude were interfered during the inhibiting activities of the active ones and also the other factors that contribute to the lack of mechanisms of its activities [30]. Hence, more studies are required to isolate the respective compounds and to determine the mechanism of action of these components.

## CONCLUSION

As a conclusion, antibacterial activities of 10 selected species of mangrove plants against *Vibrio* species were studied to identify their antibacterial activities against aquatic pathogen (*Vibrio* spp.). Among all mangrove plants screened, one species *Sonneratia caseolaris* has showed a good antibacterial and cytotoxic activity. The plant methanol extracts could inhibit five out of six *Vibrio* species tested and was less toxic to *Artemia salina* when compared to its aqueous extract. This finding indicates the presence of active compounds in methanolic extracts of *S. caseolaris* methanolic which could be further studied to produce active antibacterial compounds against aquaculture pathogenic bacteria.

## ACKNOWLEDGEMENT

The author wishes to acknowledge Faculty of Maritime Studies and Marine Science Department and Institute of Marine Biotechnology of Universiti Malaysia Terengganu for offering infrastructural facilities.

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