academicJournals

Vol. 8(8), pp. 803-813, 19 February, 2014
DOI: 10.5897/AJMR2013.6233
ISSN 1996-0808 ©2014 Academic Journals
http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Evaluation of marine macro alga, *Ulva fasciata* against bio-luminescent causing *Vibrio harveyi* during *Penaeus monodon* larviculture

Krishnamoorthy Sivakumar, Sudalayandi Kannappan*, Masilamani Dineshkumar and Prasanna Kumar Patil

Genetics and Biotechnology Unit, Central Institute of Brackishwater Aquaculture (Indian Council of Agricultural Research), 75, Santhome High Road, Raja Annamalai Puram, Chennai - 600 028, Tamilnadu, India.

Accepted 23 January, 2014

***Vibrio harveyi* is one of the major disease causing bacterium in shrimp larviculture and grow-out practices. *V. harveyi* produces many virulence cum pathogenic factors. Application of antibiotics against luminescence causes development of antibiotic resistance among *V. harveyi*. Therefore, it is obligatory to develop bio-inhibitory agents as substitute in lieu of antibiotics. Under this study, *Ulva fasciata* was collected and extracted for crude compounds, 300 µg extract showed 12.3 mm of bio-inhibition against *V. harveyi* through "agar well diffusion assay". Further, *U. fasciata* extract at 300 µg/ml was treated against *V. harveyi* in LB broth and showed reductions on phospholipase and proteolysis. Production of bio-luminescence was reduced to 7.3, 7.7, 13.0, 17.0 counts per second (CPS) and growth also reduced to 24.91%. Further, *U. fasciata* extract at 200 µg/ml was tested against *V. harveyi* during *Penaeus monodon* larviculture and showed 32.40% reduction in the cumulative percentage mortality on postlarvae due to *V. harveyi*. Chemical constituents of *U. fasciata* was characterized by FTIR and GCMS. GC-MS analysis, reported to contain organic compounds such as Bis(2-ethylhexyl) phthalate was highest (88.42%), followed by 1,2- benzenedicarboxylic acid- butyl (2.47%). Therefore, it was concluded that *U. fasciata* may be a better bio-inhibitory agent against *V. harveyi* in shrimp larviculture.**

Key words: *Ulva fasciata* extracts, antagonism, virulence factors, *Vibrio harveyi,* challenging shrimp postlarvae, cumulative mortality reduction.

INTRODUCTION

Penaeid shrimp farming have become a momentous aquaculture activity in many countries in the tropics. However, this grow-out practice is constantly under threat due to the outbreak of infectious diseases. Among the infectious diseases the luminescent disease causing *Vibrio harveyi* is one of the most important bacterial pathogen, capable of causing higher mortality among the marine invertebrates (Vezzulli et al., 2010). In the last two decades, mass mortalities (80-100%) among Penaeid shrimps resulting from *V. harveyi* infections were frequently reported in hatcheries (Raissy et al., 2011) and grow-out ponds (Zhou et al., 2012). *V. harveyi* has been established as well-known bacterium to produce extra cellular products indicating its virulence factors such as luminescence, proteases, phospholipases, lipases, siderophores, chitinases and hemolysins (Soto-Rodriguez et al., 2012). The applications of antimicrobial chemicals, especially antibiotics, led to the emergence of more virulent as well as resistant among the bacterial pathogens (Rahman et al., 2010). Under this condition, it

*Corresponding author. E-mail: sudalikanna@gmail.com. Tel: +91-44-24616948 or +91-96770 39103. Fax: +91-44-24610311.

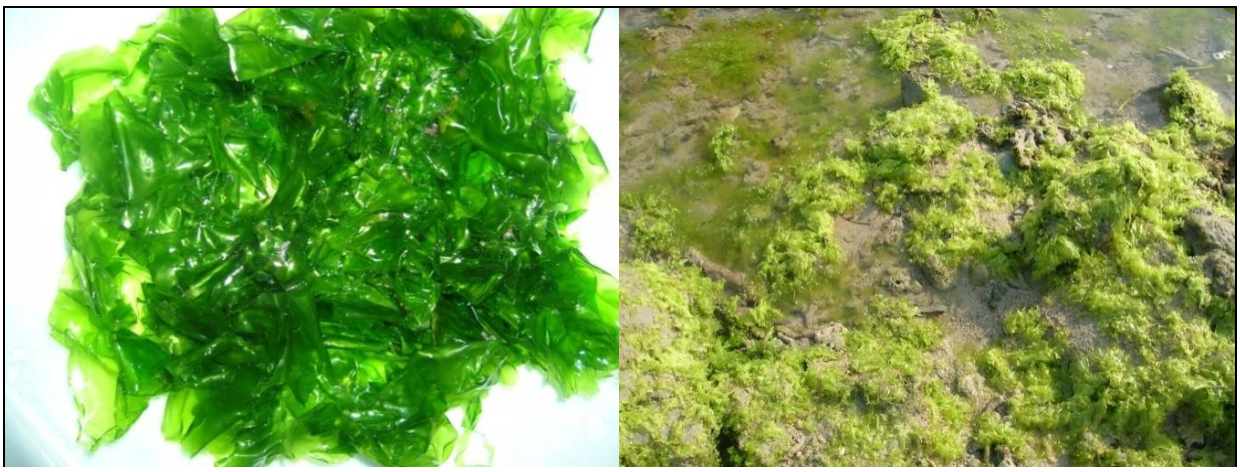

Figure 1. Marine macro alga *U. fasciata.*

is indispensible to develop an alternative agent in place of antibiotics that are commendably biodegradable and eco-friendly too.

Marine resources are an unmatched reservoir of biologically active natural products, many of which exhibit structural features that has not been found in terrestrial organism (Saritha et al., 2013). There are numerous reports on compounds derived from macro algae with a broad range of biological activities such as the anti-microbial, antiviral, anti-tumor and anti-inflammatory as well as neurotoxins (Osman et al., 2013). In addition, the macro algae derived polysaccharides for example alginate, carrageenan was capable of improving the healthiness of marine candidate fish species in aquaculture, when they were added to the diets (Peso-Echarri et al., 2012).

Current studies reported that the solvent extracts of the red seaweed *Gracilaria fisheri* prevent *V. harveyi* infections in Penaeus monodon postlarvae (Kanjana et al., 2011). The crude extract obtained from *Sargassum hemiphyllum* var. *Chinense,* show increased immunity and resistance against *Vibrio alginolyticus* and white spot syndrome virus (WSSV) infection on *Litopenaeus vannamei* (Huynh et al., 2011). *Ulva fasciata* is a green marine macro alga (*Chlorophyceae*), which grows abundantly in both intertidal and deep water regions of sea, and documented to be the potential sources of bioactive compounds (Paul and Devi, 2013). Furthermore, various extracts from *U. reticulata* and *U. lactuca* were tested for antagonism against human pathogens (Kolanjinathan and Stella, 2011). Aqueous extract of *U. fasciata* show inhibition against aquatic bacterial pathogens (Priyadharshini et al., 2012). Antimicrobial efficiency of *U. fasciata, Chaetomorpha antennina* was studied against many pathogenic bacteria (Premalatha, 2011). The efficacy of *U. fasciata harveyi* and *Aeromonas* spp. challenged with *P.monodon* tested against shrimp pathogens such as *Vibrio fischeri,* postlarvae (Selvin et al., 2011). incorporated diet was *Vibrio alginolyticus, Vibrio*

The bio-potential of marine algae such as *Skeletonema costatum*, *U. fasciata* and *Kappaphycus alvarezii* were studied against luciferase and luminescence producing *V. harveyi* (Sivakumar and Kannappan, 2013). However, numerous studies showed the biological activity of *U. fasciata* against many aquatic pathogens, but not closely determined against luminescent disease causing *V. harveyi* and its virulence factors. Thus, this study was under taken to discover the antagonistic effect of crude *U. fasciata* extract against luminescent disease causing *V. harveyi* during *P. monodon* larviculture with the, description of functional compounds by FTIR and quantification of phytochemicals by GC-MS.

MATERIALS AND METHODS

Isolation of *V. harveyi*

V. harveyi strains were isolated from the *P. monodon* larviculture tanks. The isolates were identified using standard biochemical tests and further confirmed by polymerase chain reaction (PCR) (Sivakumar and Kannappan, 2013). The pathogenicity of *V. harveyi* cells were ascertained by spotting in 3% blood agar (Hi-media, India). The isolates were re-confirmed by *V. harveyi* selective agar (VHSA) (Harris et al., 1996) and then stored in Luria-Bertani (LB) broth with sterile glycerol (15% v/v) (Hi-media, India).

Macro alga collection

The marine macro alga *U. fasciata* was collected with a knife from all over the substrate (rock, plant, wood, etc.) (Figure 1) from intertidal region of Tuticorin (Latitude 8.7874°N; Longitude 78.1983°E), Tamilnadu, India (Figure 2). The alga was washed in permanganate solution [1% $KMnO_4$ (w/v)] to remove the epiphytes, sand and other extraneous matters and then shadow dried. The dried alga was weighed, pulverised using mechanical grinder and

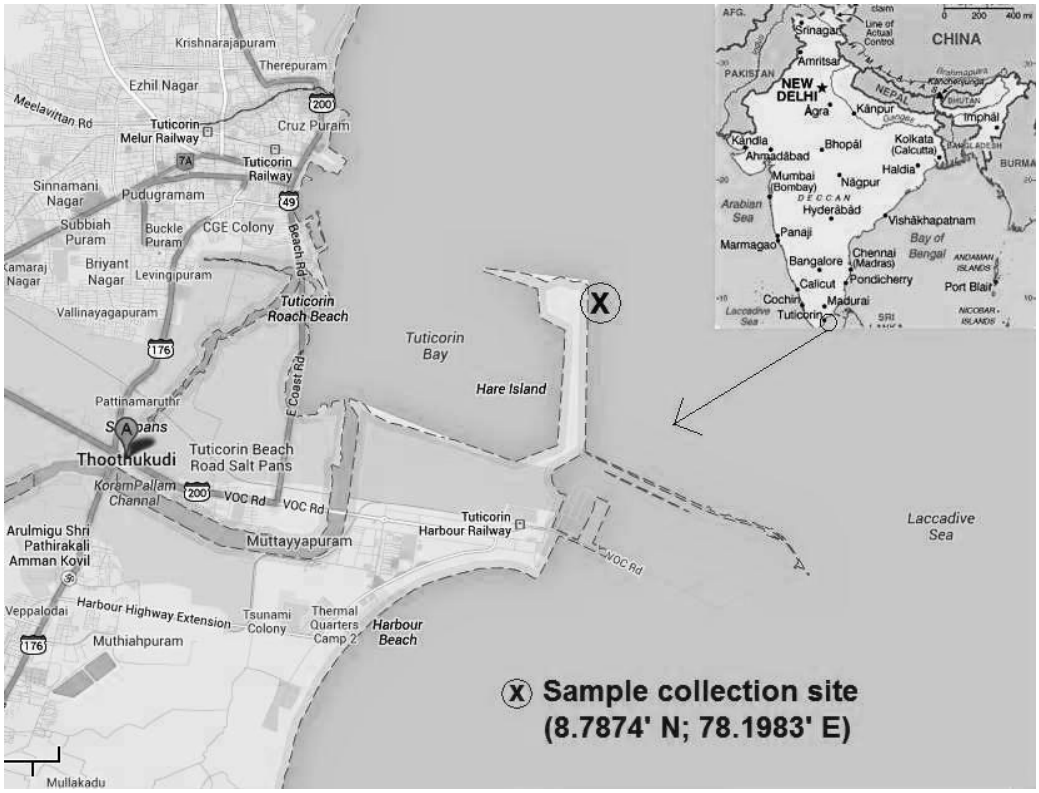

Figure 2. Map showing Tuticorin region (Latitude 8.7874°N; Longitude 78.1983°E), India where macro alga *U. fasciata* was collected.

used for extracting crude fatty acids.

Solvent extraction

Ethyl acetate was used for extracting the crude compounds from alga at 30°C called “cold extraction method”. The *U. fasciata* extract was prepared by taking 1.0 g of shadow dried powder, and then mixed with 10.0 ml of ethyl acetate and shaker incubated at 30°C for 96 h at 50 rpm. Then the extract was filtered by Whatman filter paper No. 1, rotary evaporated (30°C) under vacuum and stored at 4°C for additional use. The resultant extract was liquefied with 5 mg/ml of 30% (v/v) dimethyl sulfoxide (DMSO) and used for testing antagonism against *V. harveyi* (Sivakumar and Kannappan, 2013).

Estimation of MIC

The minimum inhibitory concentration (MIC) of *U. fasciata* against *V. harveyi* was evaluated (Islam et al., 2008).

Antibacterial assay

Antibacterial activity was ascertained against *V. harveyi* using the “agar well diffusion assay” (Sivakumar and Kannappan, 2013).

Effect of crude *U. fasciata* extract against the growth and virulence of *V. harveyi*

U. fasciata extract at 300 μg/ml was added in 100 ml of LB medium. Active 24 h old *V. harveyi* of 500 µl (1.8 OD) was inoculated into LB broth and shaker incubated at 28°C/100 rpm/5 days. The growth with various virulence factors such as luminescence, proteolytic, lipolytic, phospholipase, thermonuclease activities, crude bacteriocin production, exopolysaccharide (EPS) and protease produced by *V. harveyi* were estimated. Cell surface hydrophobicity was examined by salt aggregations test (SAT) and cell adhesion was examined by bacterial adhesion to hydrocarbons test (BATH) (Soto-Rodriguez et al., 2012). Each test was performed in triplicates and values were expressed in average with SD.

Fourier transform infra red spectroscopy (FTIR) analysis

The shadow dried *U. fasciata* was ground to powder by pestle and mortar. The FTIR spectra was recorded using BRUKER IFS 66 model FTIR spectrometer in the region 4000-400 cm^{-1} by employing standard KBr pellet technique (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 silica capillary column (length 30 m × diameter 0.25 mm × film thickness 0.25 μm) with helium at 1.51 ml for 1 min 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 injection volume was 1 µl. The injector temperature was 250°C; the oven temperature was kept at

70°C for 3 min, rose to 250°C at 14°C min^{-1} (total run time 34 min). The temperature of the transfer line and of the ion source was set to a value of 230°C and the interface temperature at 240°C, respectively. Full mass data was recorded between 50-400 Dalton per second and scan speed 2000. Mass start time is at 5 min and end time at 35 min. Peak identification of crude *U. fasciata* extract was performed 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).

Challenge of crude *U. fasciata* extract against *V. harveyi* during larviculture of *P. monodon*

The plastic tubs were washed with 1% $KMnO_4$ solution. Tubs were filled with 20 L of saline water at 20 Practical Salinity Units-PSU. Disease free postlarvae (PL 10) of *P. monodon,* procured from shrimp hatchery were acclimatized at 20 PSU for 5 days under laboratory conditions at 28 ± 1°C with continuous aeration. The average body weight of PL ranged from 17 to 18 mg and stocked at 1000 numbers per each tub. The control tub was inoculated with *V. harveyi* (10 ml of 1.80 OD) alone. Second tub was considered as treatment inoculated with *V. harveyi* and 200 µg (2 gm/10L) of crude *U. fasciata* extract per ml. Third tub was considered as control where crude *U. fasciata* extract was added at 200 µg per ml alone with PL. The fourth tub was a control for PL, where neither *V. harveyi* nor extract was added. The aeration was given for each tub to provide oxygen level not more than 4 ppm. The PL feed was given twice at 15% of their body weight. The water quality parameters such as temperature, salinity and pH were measured once in 5 days. The mortality of PL was counted daily. No water exchange was given for all the tubs till 30 days. The water samples were collected once in 5 days by sterile water bottles. The total heterotrophic and *V. harveyi* bacterial counts were enumerated using LB medium and *V. harveyi* selective agar medium under spread plate method. All the experimental tubs were top covered to avoid any external contaminations. For each experiment, triplicates were maintained and the values are average of three determinations (Traifalgar et al., 2009; Kannappan et al., 2013).

RESULTS

Minimum inhibitory concentration (MIC) of *U. fasciata*

The MIC of crude extracts of *U. fasciata* was established at 30 µg concentration. The zone of inhibition was 12.3 mm, established at 300 µg level of extract of *U. fasciata* whereas, the 200 and 100 µg level of concentrations showed 8.3 and 3.3 mm zones, respectively, against the growth of *V. harveyi*.

Effect of crude extract of *U. fasciata* against the changes in growth and virulence factors produced by *V. harveyi*

The treatment reduced the growth of *V. harveyi* (1.8 OD) from 1st to 5th day. The highest growth differences was observed on 3rd day (0.532 OD) and lowest on 1st and 5th day (0.468 OD) as compare to the control (Figure 3a). The maximum reduction on bacteriocin production (OD) was on 4th and 5th day (0.177 and 0.184) and minimum (0.064) observed on 1st day as compared to the control (OD 1.986 and 1.978 on 4th and 5th, and 2.082 on 1st days). Although, reductions of crude extra cellular protein released was noticed in all the treatment days (Figure 3b). The EPS production in the treatment was reduced to 0.525, 0.556, 0.585, 0.551 and 0.507 from 1st to 5th days as compared to the control (OD 2.026, 2.408, 2.242, 2.262 and 2.250) (Figure 3c). The protease level was reduced from 0.047 and 0.051 as compared to the control (OD 0.146 and 0.171) (Figure 3d).

Further, the treated *V. harveyi* cells were subjected to phospholipase, proteolysis, lipolysis and thermonuclease activities, determined based on the hydrolysis of medium in the plate assay (Table 1). The activities were coded with qualitative parameters like weak, moderate, high and very high. In treatment, the moderate level of phospholipase and proteolysis activity was noticed on 1st, 2nd and 3rd days. Weak activities were noticed on 4th and 5th days (very high). But moderate level of lipolysis and thermonuclease activities was shown on 1st to 5th day as compared to the control (very high).

Cell surface hydrophobicity was examined using SAT and BATH tests (Table 1). SAT test was determined as the lowest molarity of ammonium sulphate (0.05-4.0 M) that caused visible agglutination of a test organism. In SAT test, the control *V. harveyi* revealed strong hydrophobic activity for 1st to 5th day whereas, the treated showed moderate hydrophobic activity for 1st to 5th day. Similar way, BATH test also exhibited strong level of hydrophobic activity for control from 1st to 5th day. When crude extract of macro alga of *U. fasciata* was treated with *V. harveyi*, the production on luminescence was reduced to 7.3, 7.7, 13.0 and 17.0 CPS (counts per second) for the 4 days period (Figure 3e). The maximum reduction on luminescence was reported on 4thday (17.0 CPS) and minimum was observed on the 1st day (7.3 CPS) when compared with the control (39.6, 50.3, 59.3, 63.6 CPS).

FTIR of *U. fasciata*

The FTIR spectrum of dried powder of *U. fasciata* is shown in Figure 4 and functional groups identified were compared from the FTIR standard library data. FTIR spectrum showed the presence of significant functional groups such as alcohols, phenols, esters, ethers, alkanes, alkenes, primary amines, nitro compounds, aromatics and carboxylic acids, alkyl halides and aliphatic amines, etc (Table 2).

GC-MS of *U. fasciata*

GC-MS analysis of crude ethyl acetate extract of *U.*

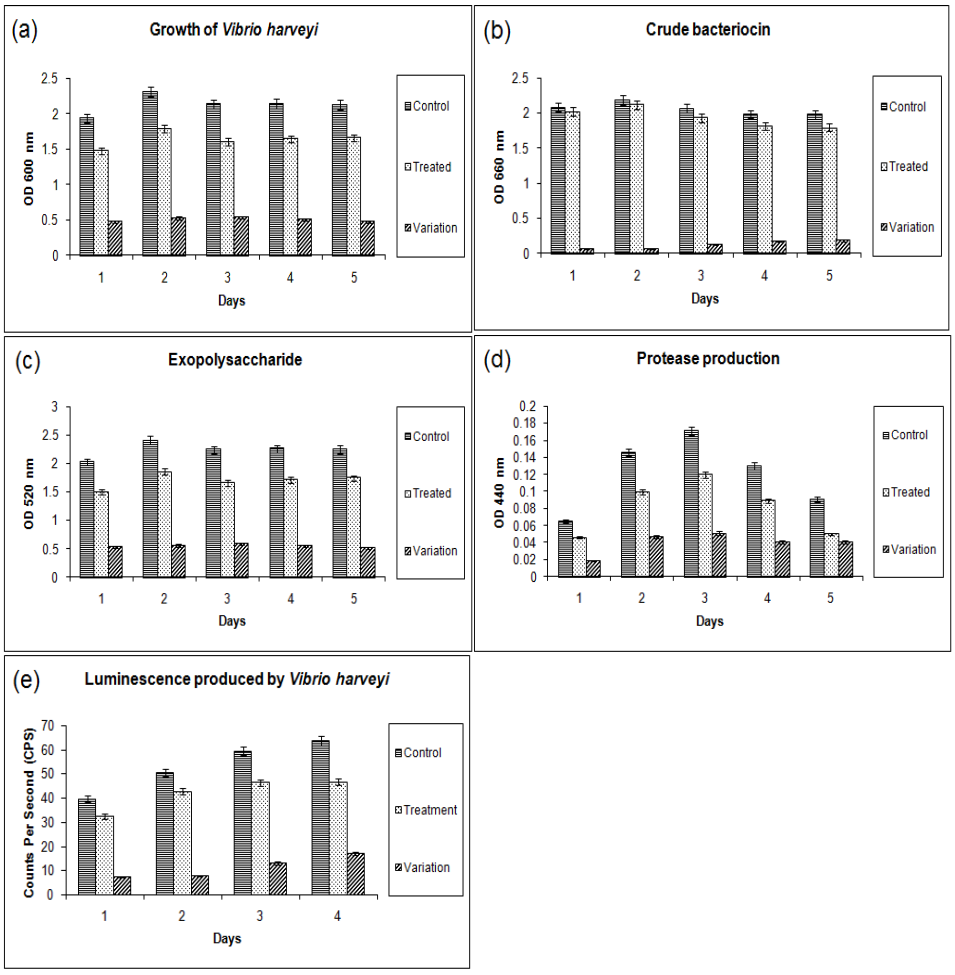

Figure 3. Crude extract of *U. fasciata* against the changes of growth and virulences produced by *V. harveyi* in LB broth for 5 days.

fasciata was found to have mixture of volatile compounds. A total of 36 peaks were observed with retention times as shown in Figure 5. Chemical constituents were identified using spectrum data base NIST 11 software installed in GC-MS.

The GC-MS analysis of the crude extract revealed that the main chemical-constituent was organic compound Bis(2-ethylhexyl) phthalate (tR = 23.21 min) (88.42 %) followed by 1,2-benzenedicarboxylic acid- butyl (tR = 18.14 min) (2.47%) (Figure 6a and b). It is possible that bioactive compounds primarily consisting of Bis(2-ethylhexyl)phthalate (tR =23.21 min) (88.42%) may be involved in biological activity (Table 3) with other compounds.

Challenge of crude *U. fasciata* extract against *V. harveyi* during *P. monodon* larviculture

When *U. fasciata* extract was tested against *V. harveyi* in *P. monodon* postlarvae for 30 days, the reduction on cumulative percentage of mortality on postlarvae was noticed as 32.40% as compared to the control (76.30%). Two trails were maintained under larviculture as negative controls to distinguish any influence of *U. fasciata* extract on PL. However, it was noticed that treatment does not affect PL as compared to the control (76.30%) which showed less reduction on cumulative percentage mortality with extract and PL (29.56%) and with PL alone (28.39%). The weight of the PL was measured for both the control and treatments. There was no much weight difference observed both in the treatment and control. On 30^{th} day, the average weight of the PL was 269.3 and 266.5 mg for control and treatment, respectively (initial weight of the PL for control was 17.7 and 18.1 mg for treatment, respectively).

The maximum decrease on *V. harveyi* counts were observed on 5^{th}, $10^{th,}$ 15^{th} and 20^{th} days and mean values for treatment were 2.38×10^4, 1.56×10^4, 4.30×10^3 and

Table 1. Effect of *U. fasciata* extract against the changes of virulences produced by *V. harveyi.*

Day	Virulence studied											
	Proteolysis		Phospholipase		Lipolysis		Thermonuclease		Cell surface hydrophobicity			
									SAT (M)		BATH (%)	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated
1	++++	++	++++	++	++++	++	++++	++	0.86± 0.02	1.29± 0.04	86.78±4.11	46.33±2.13
2	++++	++	++++	++	++++	++	++++	++	0.89± 0.03	1.35± 0.05	85.66±3.91	43.11 ±1.65
3	++++	++	++++	++	++++	++	++++	++	0.91± 0.04	1.44± 0.06	82.33± 3.03	39.56± 1.33
4	++++	+	++++	+	++++	++	++++	++	0.91± 0.03	1.49± 0.04	76.31±3.13	36.81± 1.29
5	++++	+	++++	+	++++	++	++++	++	0.99± 0.04	1.55± 0.06	73.46± 2.96	36.31± 1.19

Control- *V. harveyi* untreated with crude extract; Treated- *V. harveyi* treated with crude extract of *U. fasciata*; Activity of *V. harveyi* + = weak; ++ = moderate; +++ = high; ++++ = very high; SAT test (0.0 to 1.0 molarity (M) = strongly hydrophobic, 1.0 to 2.0 M = moderately hydrophobic; 2.0 to 4.0 M = weakly hydrophobic, and >4.0 M = not hydrophobic); BATH-test (>50% partitioning = strongly hydrophobic, 20 to 50% partitioning = moderately hydrophobic; and <20 % partitioning = not hydrophobic).

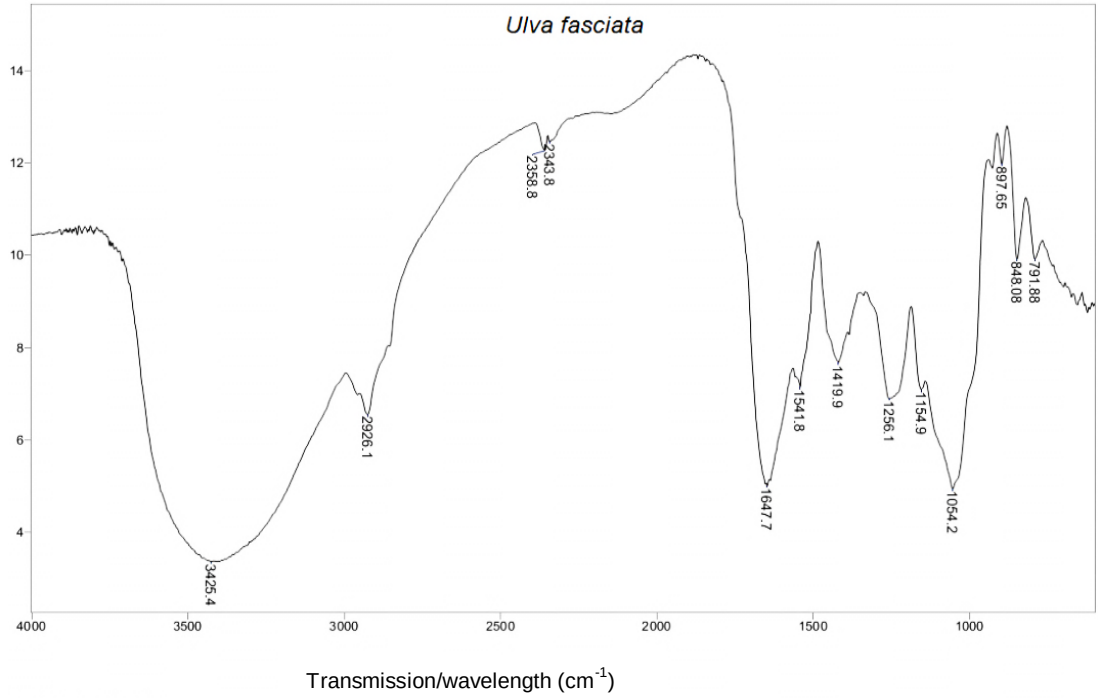

Figure 4. FTIR spectrum of shadow dried powder of *U. fasciata.*

Table 2. The wave number (cm^{-1}) of dominant peak obtained from the FTIR absorption spectra of *U. fasciata.*

Frequency (cm^{-1})	Bond	Functional groups
3425.4	O-H stretch, H-bonded	Alcohols, phenols
2926.1	O-H stretch	Carboxylic acids
	C-H stretch	Alkanes
1647.7	-C=C- stretch	Alkenes
	N-H bend	Primary amines
1541.8	N-O asymmetric stretch	Nitro compounds
1419.9	C-C stretch (in-ring)	Aromatics
1256.1	C-N stretchC-O stretch	Aromatic amines
		Alcohols, carboxylic acids, esters, ethers
	C-H wag (-CH_2X)	Alkyl halides
1154.9	C-O stretch	Alcohols, carboxylic acids, esters, ethers
	C-H wag (-CH_2X)	Alkyl halides
	C-N stretch	Aliphatic amines
1054.2	C-N stretch	Aliphatic amines
897.65	=C-H bend	Alkenes
	C-H "oop"	Aromatics
	N-H wag	Primary, secondary amines
848.08	=C-H bend	Alkenes
	N-H wag	Primary, secondary amines
	C-H "oop"	Aromatics
	C-Cl stretch	Alkyl halides
791.88	=C-H bend	Alkenes
	N-H wag	Primary, secondary amines
	C-H "oop"	Aromatics
	C-Cl stretch	Alkyl halides

3.40×10^3 cfu/ml as compared to control which is 3.40×10^5, 1.44×10^5, 1.45×10^5 and 2.49×10^4 cfu/ml, respectively. Various water quality parameters like temperature, salinity and pH were observed in every sampling are presented in Table 4. There were not much changes of water quality parameters both in the treatment and control. Although, in the treatment, and with extract alone, light greenish coloration was observed when compared with control due to the crude nature of extract.

DISCUSSION

In the present study, *U. fasciata* extract reduced the growth of *V. harveyi.* The preliminary phytochemical characterization and antimicrobial efficacy of macro algae *U. fasciata* and *Chaetomorpha antennina* were studied against pathogenic bacteria (Premalatha, 2011). Priyadharshini et al. (2012) observed that aqueous and solvent based extracts of *U. fasciata* showed inhibition against fish-borne bacteria and fungal pathogens. Kolanjinathan and Stella (2011) reported that crude extracts of *U. reticulata* and *U. lactuca* are inhibitory to human pathogenic bacteria and fungi. The dietary administration of *U. fasciata* extracts controlled marine *V. harveyi* in shrimp grow-out system (Selvin et al., 2011).

In a recent study, reductions on crude bacteriocin were noticed in all the days by *U. fasciata* extract on *V. harveyi*. The moderate and weak levels of reduction on proteolysis, phospholipase, lipolysis and thermonuclease of *V. harveyi* were observed treating against *U. fasciata* extract. Silva et al. (2013a) has observed that *U. fascita* extract exhibited antagonism against *V. parahaemolyticus.*

In this study, the cell surface hydrophobicity of *V. harveyi* exhibit moderate hydro-phobicity by *U. fasciata* treatment when compared with the control. This was corroborated with the values reported by Sivakumar and Kannappan

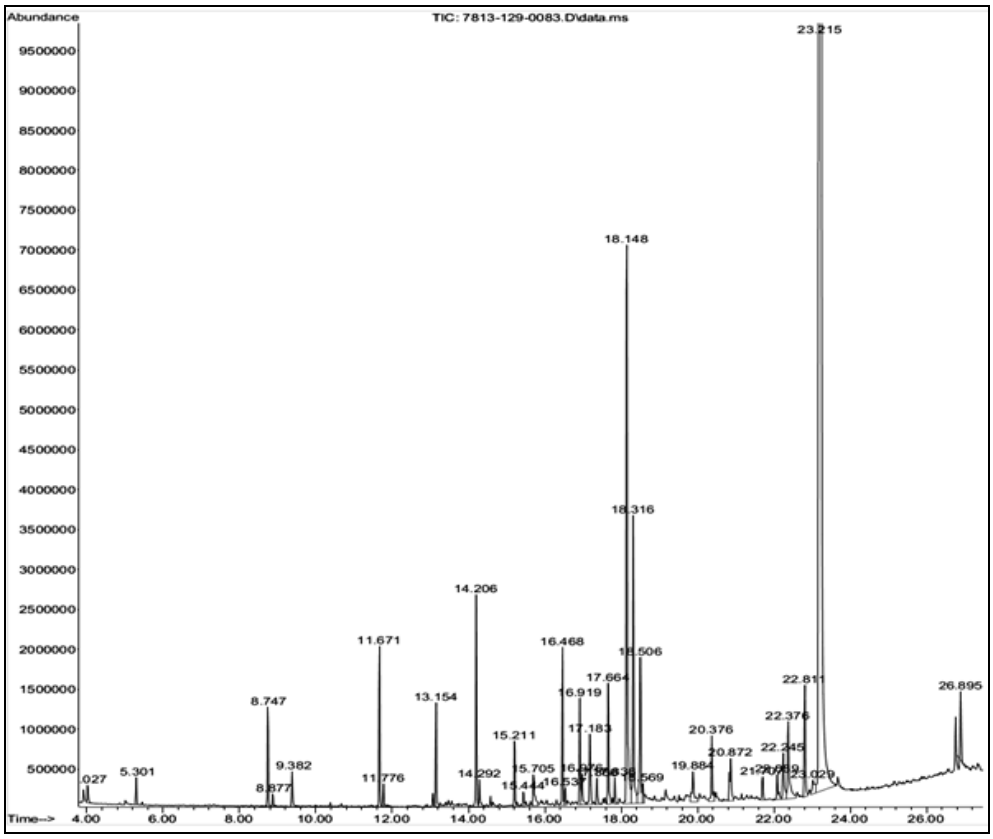

Figure 5. GC-MS chromatogram of the crude ethyl acetate extract of *U. fasciata.*

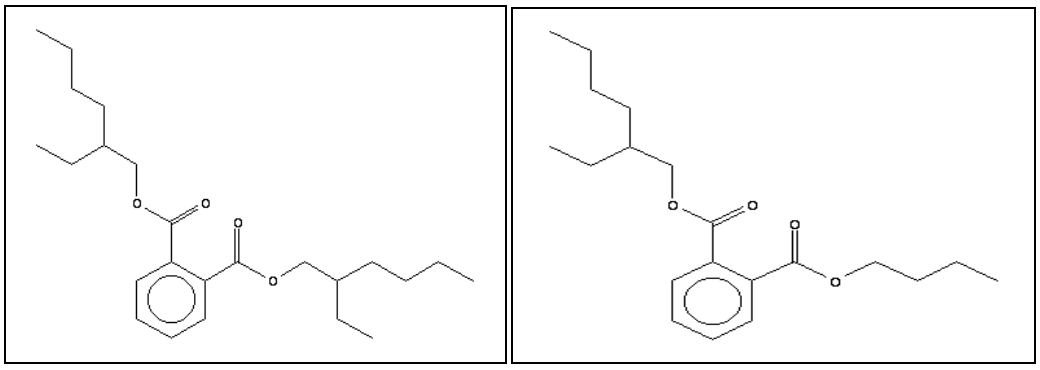

Figure 6. Major compounds isolated from *U. fasciata*. (a) Structure of Bis(2-ethylhexyl)phthalate ($C_{24}H_{38}O_4$) extracted and detected by GC-MS from *U. fasciata* (b) Structure of 1,2-benzenedicarboxylic acid, butyl 2-ethylhexyl ester ($C_{20}H_{30}O_4$), extracted and detected by GC-MS from *U. fasciata*.

(2013) from the marine algae such as *S. costatum* and *K. alvarezii.*

The FTIR spectra of *U. fasciata* showed various functional groups of compounds which agreed with the FTIR values reported for marine macro algae *Laminaria digitata* (Dittert et al., 2012). Similarly, Azizi et al. (2013) observed various functional compounds like water, protein, polysaccharide and lipids from marine algae *Sargassum muticum* using FTIR. Though the GC-MS analysis of crude extract of *U. fasciata* revealed many components, the main chemical constituents observed in high percentage were Bis(2-ethylhexyl) phthalate and 1,2-benzenedicarboxylic acid-butyl which may also be involved in antagonism against *V. harveyi*.

Challenge against *V. harveyi* during *P. monodon* post-larvae revealed that *U. fasciata* extract showed 32.40%

Table 3. GC-MS profile of *U. fasciata.*

Retention time (min)	Compound's name	Peak area (%)	Molecular formula	Molecular weight
4.03	Anisole	0.80	C_7H_8O	108.13
5.29	1-Decene	0.09	$C_{10}H_{20}$	140.26
8.75	5-Tetradecene, (E)-	0.34	$C_{12}H_{28}$	196.37
8.88	Dodecane	0.05	$C_{12}H_{26}$	170.33
9.37	Benzothiazole	0.14	C_7H_5NS	135.18
11.67	1-Tetradecene	0.52	$C_{14}H_{28}$	196.37
11.77	Tetradecane	0.07	$C_{14}H_{30}$	198.38
13.15	Phenol, 2,4-bis(1,1-dimethylethyl)	0.34	$C_{14}H_{22}O$	206.32
14.21	Cetene	0.60	$C_{16}H_{32}$	224.42
14.29	Hentriacontane	0.10	$C_{31}H_{64}$	436.83
15.21	8-Heptadecene	0.23	$C_{17}H_{34}$	238.45
15.44	Heptadecane	0.06	$C_{17}H_{36}$	240.46
15.70	Phenol, 2-(1-phenylethyl)-	0.19	$C_{14}H_{14}O$	198.26
16.46	1-Octadecene	0.47	$C_{18}H_{36}$	252.48
16.53	Octadecane	0.06	$C_{18}H_{38}$	254.49
16.91	Bicyclo[3.1.1]heptanes, 2,6,6-trimethyl-,(1.alpha.,2.beta.,5.alpha)	0.32	$C_{10}H_{18}$	138.24
16.97	2-Undecanone, 6,10-dimethyl-	0.13	$C_{13}H_{26}O$	198.34
17.18	Dibutyl phthalate	0.30	$C_{16}H_{22}O_4$	278.34
17.36	Phytol, acetate	0.11	$C_{22}H_{42}O_2$	338.56
17.66	Phthalic acid, butyl isohexyl ester	0.45	$C_{18}H_{26}O_4$	306.39
17.84	Phthalic acid, 2-ethylhexyl pentyl ester	0.09	$C_{21}H_{32}O_4$	348.47
18.14	1,2-Benzenedicarboxylic acid, butyl	2.47	$C_{20}H_{30}O_4$	334.44
18.32	Phthalic acid, butyl isohexyl ester	1.12	$C_{18}H_{26}O_4$	306.39
18.51	5-Eicosene, (E)-	0.66	$C_{20}H_{40}$	280.53
18.56	Dodecane, 1,1'-oxybis-	0.10	$C_{24}H_{50}O$	354.65
19.89	Silanetriamine,1-azido-N,N,N',N', N'',N''-hexamethyl-	0.21	$C_6H_{18}N_6Si$	202.33
20.36	Behenic alcohol	0.26	$C_{22}H_{46}O$	326.60
20.87	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	0.27	$C_{20}H_{30}O_4$	334.44
21.70	Methyl dehydroabietate	0.10	$C_{21}H_{30}O_2$	314.46
22.08	Octacosanol	0.12	$C_{28}H_{58}O$	410.75
22.24	Phenol, 2,4-bis(1-phenylethyl)-	0.25	$C_{30}H_{29}N_3O_3$	479.56
22.37	Phenol, 2,4-bis(1-phenylethyl)-	0.49	$C_{30}H_{29}N_3O_3$	479.56
22.80	Bis(2-ethylhexyl) phthalate	0.45	$C_{24}H_{38}O_4$	390.55
23.02	Naphthalene, 6-chloro-1-nitro-	0.13	$C_{10}H_6ClNO_2$	207.61
23.21	Bis(2-ethylhexyl) phthalate	88.42	$C_{24}H_{38}O_4$	390.55
26.89	Benzo[h]quinoline, 2,4-dimethyl-	0.22	$C_{15}H_{13}N$	207.27

reduction in the cumulative percentage of mortality as compared to control; but, Saptiani et al. (2011) has reported that ethyl acetate, n-butanol fractions of crude *Acanthus ilicifolius* extract controlled *P. monodon* postlarvae from *V. harveyi* infections. The supplementation of *Undaria pinnatifida* and fucoidon incorporated diet proved to enhance growth with reduced mortalities among *P. monodon* postlarvae caused by *V. harveyi* (Traifalgar et al., 2009). Marine algae are impending source for owning extensive range of polyunsaturated fatty acids (PUFA), carotenoids, phycobiliproteins, polysaccharides and phycotoxins, etc (Chu, 2012). It was reported that lipids obstruct microbesby distracting cellular membrane (Bergsson et al., 2011) of bacteria, fungi and yeasts. These fatty acids may further distress the expression of bacterial virulence which was significant for establishing infections.

Table 4. Challenging of crude extracts of *U. fasciata* against *V. harveyi* during *P. monodon* larviculture with the cumulative percentage mortality reduction.

Day	Cumulative percentage mortality				Treatment tubs (cfu/ml)		Control tubs (cfu/ml)		Average weight of postlarvae (mg)		Water quality parameters for treatment and control tubs			
	Control tubs with *V. harveyi*	Treatment tubs extract with *V. harveyi*	Tubs with extract and PL alone	Tubs with PL alone	Total plate count	*V. harveyi*	Total plate count	*V. harveyi*	Treatment tubs	Control tubs	Temperature (°C)	Salinity (PSU)	pH in control tubs	pH in treatment tubs
0	0.00	0.00	0.00	0.00	1.24×10^{6}	1.15×10^{6}	1.18×10^{6}	2.41×10^{6}	17.7 ± 3	18.1±2	29.0±1.0	20±0.5	8.40±0.2	8.30±0.2
5th	13.66±0.3	06.96±0.2	2.39±0.1	3.23±0.1	2.42×10^{4}	2.38×10^{4}	3.18×10^{5}	3.40×10^{5}	60.9 ± 4	63.6±3	29.5±1.0	20±0.5	8.50±0.2	8.40±0.2
10th	26.05±0.9	14.36±0.3	6.19±0.2	6.03±0.2	2.15×10^{4}	1.56×10^{4}	2.94×10^{5}	1.44×10^{5}	121.1 ± 4	127.5±5	29.0±1.0	20±0.5	8.20±0.2	8.30±0.2
15th	35.63±1.1	21.33±0.6	12.05±0.5	13.33±0.5	7.40×10^{4}	4.30×10^{3}	1.51×10^{5}	1.45×10^{5}	156.3 ± 5	157.5±5	30.0±1.0	20±0.5	8.40±0.2	8.50±0.2
20th	47.33±1.5	27.81±1.1	18.13±0.6	17.43±0.5	1.29×10^{4}	3.40×10^{3}	2.66×10^{4}	2.49×10^{4}	201.5 ± 9	197.9±7	30.0±1.0	21±0.5	8.10±0.2	8.30±0.2
25th	62.13±2.3	36.63±1.3	24.69±0.9	23.86±1.0	9.40×10^{4}	5.40×10^{3}	1.80×10^{4}	1.74×10^{4}	236.9 ± 8	240.1±9	31.0±1.0	21±0.5	8.40±0.2	8.20±0.2
30th	76.30±2.9	43.90±1.3	29.56±1.0	28.39±1.0	8.20×10^{4}	4.10×10^{3}	1.74×10^{4}	1.51×10^{4}	269.3 ± 9	266.5±8	30.0±1.0	21±0.5	8.10±0.2	8.00±0.2

Values of average of three determinations with standard deviation (SD)

It has been demonstrated that fatty acids of chain length more than 10 carbon atoms would induce lysis of bacterial protoplasts. Due to the tough environments in which many macro algae exists, effective defense mechanisms have been established and consequently, amusing source of bioactive compounds, including polysaccharides, polyphenols, fatty acids and peptides, with dissimilar structures and activities than those found in terrestrial plants (Tierney et al., 2010).

Many species of macro algae had foremost constituentslike tetradecanoic acid, hexadecanoic acid, octadecanoic acid methyl esters etc (Balamurugan et al., 2013) which may reveal antagonism against marine bacteria (Al-Saif et al., 2013). Lately, the secondary metabolites and organic extracts obtained from *U. fasciata* has potential applications (Silva et al., 2013b) and the diverse derivatives of diterpenoids extracted from *U. fasciata* exhibited antagonism against *Vibrio parahaemolyticus* and *V. harveyi* (Chakraborty et al., 2010). Thus, in the present study, biological activity of *U. fasciata* against *V. harveyi* was due to the presence of various chemical constituents as described. Hence, macro algae *U. fasciata* extract will have immense applications in aquaculture.

Conclusion

Results from this study proved that the crude extract of *U. fasciata* at 300 µg/ml inhibited the growth and modulated the virulences produced by *V. harveyi*. *U. fasciata* extract at 200 µg/ml also controlled the mortality caused by *V. harveyi* during shrimp larviculture. Based on this study, the *U. fasciata* extract can be used as alternative bio-inhibitors for the aquaculture practices. Application of such bio-products would moderate the undesirable contamination from applying the synthetic compounds with reduced cost and eco-friendly nature.

ACKNOWLEDGEMENTS

The authors greatly acknowledge the Department of Biotechnology (DBT), Government of India for sponsoring the project entitled “Development of inhibitors for controlling quorum sensing luminescence disease causing *Vibrio harveyi* in shrimp larviculture system” during 2010. This work was a component under this project (BT/PR/13383/AAQ/03/501/2009).

REFERENCES

Al-Saif SSAL, Abdel-Raouf N, El-Wazanani HA, Aref IA (2013). Antibacterial substances from marine algae isolated from Jeddah coast of Red Sea, Saudi Arabia. Saudi J. Biological Sci. (article in press).

Azizi S, Namvar F, Mahdavi M, Ahmad MB, Mohamad R (2013). Biosynthesis of silver nanoparticles using brown marine macro alga, *Sargassum muticum* aqueous extract. Materials 6:5942-5950.

Balamurugan M, Selvam GG, Thinakaran T, Sivakumar K (2013). Biochemical Study and GC-MS Analysis of *Hypnea musciformis* (Wulf) Lamouroux. American-Eurasian J. Sci. Res. 8(3):117-123.

Bergsson G, Hilmarsson H, Thormar H (2011). Antibacterial, antiviral and antifungal activities of lipids. In: Thormar H. (eds), Lipids and essential oils as antimicrobial agents. John Wiley and Sons Limited, Chichester, United Kingdom. pp. 47-80.

Chakraborty K, Lipton AP, Paulraj R, Vijayan KK (2010). Antibacterial labdanediterpenoidsof *Ulva fasciata* Delile from southwestern coast of the Indian Peninsula. Food Chem. 119(4):1399-1408.

Chu WL (2012). Biotechnological applications of microalgae. IeJSME 6(1):S24-S37.

Dittert I M, Vilar VJP, da Silva EAB, de Souza SMAGU, de Souza AAU, Botelho CMS, Boaventura RA (2012). Adding value to marine macro algae *Laminaria digitata* through its use in the separation and recovery of trivalent chromium ions from aqueous solution. Chem. Eng. J. 193:348-357.

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.

Harris L, Owens L, Smith S (1996). A selective and differential medium for *Vibrio harveyi*. Appl. Enviorn. Microbiol. 62(9):3548-3550.

Huynh TG, Yeh ST, Lin YC, Shyu JF, Chen LL, Chen JC (2011). White shrimp *Litopenaeus vannamei* immersed in seawater containing *Sargassum hemiphyllum* var. *Chinense* powder and its extract showed increased immunity and resistance against *Vibrio alginolyticus* and white spot syndrome virus. Fish Shellfish Immunol. 31(2):286-93.

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.

Kanjana K, Radtanatip T, Asuvapongpatana S, Withyachumnarnkul B, Wongprasert K (2011). Solvent extracts of the red seaweed *Gracilaria fisheri* prevent *V. harveyi* infections in the black tiger shrimp *Penaeus monodon*. Fish Shellfish Immunol. 30(1):389-396.

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

Kolanjinathan K, Stella D (2011). Comparative studies on antimicrobial activity of *Ulva reticulata* and *Ulva lactuca* against human pathogens. Int. J. Pharma. Biol. Archives 2(6):1738-1744.

Musharraf SG, Ahmed MA, Zehra N, Kabir N, Choudhary MI, Rahman AU (2012). Biodiesel production from micro algal isolates of southern Pakistan and quantification of FAMEs by GC-MS/MS analysis. Chem. Cent. J. 6(1):1-10.

Osman MEH, Aboshady AM, Elshobary ME (2013). Production and characterization of antimicrobial active substance from some macro algae collected from Abu- Qir bay (Alexandria) Egypt. Afr. J. Biotechnol. 12(49):6847-6858.

Paul JJP, Devi SDKS (2013). Seasonal variability of *Ulva* species (Green seaweed) in Tirunelveli region, the southeast coast of Tamil Nadu, India. Res. J. Marine Sci. 1(1):14-17.

Peso-Echarri P, Frontela-Saseta C, Gonzalez-Bermudez CA, Ros-Berruezo GF, Martinez-Gracia C (2012). Polysaccharides from seaweed as ingredients in marine aquaculture feeding: alginate, carrageenan and ulvan. Revista de Biologia Marina y Oceanografia 47(3):373-381.

Premalatha M (2011). Phytochemical characterization and antimicrobial efficiency of seaweed samples, *Ulva fasciata* and *Chaetomorpha antennina.* Int. J. Pharma Bio Sci. 2(1):P288-293.

Priyadharshini S, Bragadeeswaran S, Prabhu K, Sophia Rani S (2012). Antimicrobial and hemolytic activity of seaweed extracts *Ulva fasciata* (Delile 1813) from Mandapam, Southeast coast of India. Asian Pac. J. Trop. Biomed. S38-S39.

Rahman S, Khan SN, Naser MN, Karim MM (2010). Isolation of *Vibrio* spp. from Penaeidshrimp hatcheries and coastal waters of Cox's Bazar, Bangladesh. Asian J. Exp. Biol. Sci. 1(2):288-293.

Raissy M, Momtaz H, Moumeni M, Ansari M, Rahimi E (2011). Molecular detection of *Vibrio* spp. in lobster haemolymph. Afri. J. Microbiol. Res. 5(13):1697-1700.

Saptiani G, Prayitno SB, Anggoro S (2011). Inhibition test of jeruju (*Acanthus ilicifolius*) leaf extracts on the *in vitro* growth of the *Vibrio harveyi*. Paper. Simposium Nasional Kimia Bahan Alam XIX. Simnas KBA. Samarinda 11-12 p October (In Indonesian).

Saritha K, Mani AE, Priyalaxmi M, Patterson J (2013). Antibacterial activity and biochemical constituents of seaweed *Ulva lactuca*. Global J. Pharmacol. 7(3):276-282.

Selvin J, Manilal A, Sujith S, Kiran GS, Lipton AP (2011). Efficacy of marine green alga *Ulva fasciata* extracts on the management of shrimp bacterial diseases. Lat. Am. J. Aquat. Res. 39(2):197-204.

Silva GC, Albuquerque-Costa R, Oliveira-Peixoto JR, Macedo-Carneiro PBD, Fernandes-Vieira RHSD (2013a).Tropical Atlantic marine macro algae with bioactivity against virulent and antibiotic resistant *Vibrio*. Lat. Am. J. Aquat. Res. 41(1):183-188.

Silva M, Vieira L, Almeida AP, Kijjoa A (2013b). The marine macro algae of the genus *Ulva*: chemistry, biological activities and potential applications. Oceanography 1:101. doi:10.4172/ocn.1000101.

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*. Afri. 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 *Vibrio harveyi* responsible for the "Bright-red" Syndrome in the Pacific white shrimp *Litopenaeus vannamei.* J. Invertebrate Pathol. 109(3):307-317.

Tierney MS, Croft AK, Hayes M (2010). A review of antihypertensive and antioxidant activities in macro algae. Botanica Marina 53(5):387-408.

Traifalgar RF, Serrano AE, Corre V, Kira H, Tung HT, Fady RM, Kader Md. A, Laining A, Yokoyama S, Ishikawa M, Koshio S (2009). Evaluation of dietary fucoidan supplementation effects on growth performance and *Vibriosis* resistance of *Peneaus monodon* postlarvae. Aquaculture Sci. 57:167-174.

Vezzulli L, Previati M, Pruzzo C, Marchese A, Bourne DG, Cerrano C (2010). *Vibrio* infections triggering mass mortality events in a warming Mediterranean Sea. Environ. Microbiol. 12:2007-2019.

Zhou J, Fang W, Yang X, Zhou S, Hu L, Li X, Qi X, Su H, Xie L (2012). A non-luminescent and highly virulent *V. harveyi* strain is associated with ''bacterial white tail disease'' of *Litopenaeus vannamei* shrimp. PLoS One 7(2): e29961.