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.

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*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-buty (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...
is indispensable 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 antimicrobial, 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 alvarezi 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.
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% KMnO₄ 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 and the 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 4th day (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, amines, primary amines, nitrates, compounds, aromatics and hydroxylic acids, aliphatic amines, etc (Table 2).

**GC-MS of U. fasciata**

GC-MS analysis of crude ethyl acetate extract of U.
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 30th 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 5th, 10th, 15th and 20th days and mean values for treatment were 2.38 x 10^4, 1.56 x 10^4, 4.30 x 10^3 and
Table 1. Effect of *U. fasciata* extract against the changes of virulences produced by *V. harveyi*.

| Day | Proteolysis | Phospholipase | Lipolysis | Thermonuclease | Cell surface hydrophobicity |
|-----|-------------|---------------|-----------|----------------|----------------------------|
|     | 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).

![FTIR spectrum](attachment:ulva-fasciata-ftir.png)

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

*Transmission/wavelength (cm$^{-1}$)*
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=O- 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 stretch, C-O stretch    | Alcohols, carboxylic acids, esters, ethers             |
|                 | H wag (-CH₂X)               | Alkyl halides                                         |
| 1154.9          | C-O stretch                 | Alcohols, carboxylic acids, esters, ethers             |
|                 | H wag (-CH₂X)               | Alkyl halides                                         |
|                 | C-N stretch                 | Aliphatic amines                                      |
| 1054.2          | =C-H bend                   | Alkenes                                               |
| 897.65          | C-H "oop"                   | Aromatics                                             |
|                 | N-H wag                     | Primary, secondary amines                             |
|                 | =C-H bend                   | Alkenes                                               |
| 848.08          | N-H wag                     | Primary, secondary amines                             |
|                 | C-H "oop"                   | Aromatics                                             |
|                 | C-Cl stretch                | Alkyl halides                                         |
|                 | =C-H bend                   | Alkenes                                               |
| 791.88          | N-H wag                     | Primary, secondary amines                             |
|                 | C-H "oop"                   | Aromatics                                             |
|                 | C-Cl stretch                | Alkyl halides                                         |

3.40 x 10³ cfu/ml as compared to control which is 3.40 x 10⁵, 1.44 x 10⁵, 1.45 x 10⁵ and 2.49 x 10⁴ cfu/ml, respectively. Various water quality parameters like temperature, salinity and pH were observed in every sampling are presented in Table 4. There were no 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. Kolanjinnathan 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. fasciata 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          | C10H18            | 138.24           |
| 5.29                 | 1-Decene        | 0.09          | C10H20            | 140.26           |
| 8.75                 | 5-Tetradecene, (E)- | 0.34    | C12H28            | 196.37           |
| 8.88                 | Dodecane        | 0.05          | C12H26            | 170.33           |
| 9.37                 | Benzothiazole   | 0.14          | C6H5NS            | 135.18           |
| 11.67                | 1-Tetradecene   | 0.52          | C13H30            | 198.38           |
| 11.77                | Tetradecane     | 0.07          | C14H32            | 224.42           |
| 13.15                | Phenol, 2,4-bis(1,1-dimethylethyl) | 0.34 | C14H25O | 206.32 |
| 14.21                | Cetene          | 0.60          | C16H32            | 246.46           |
| 14.29                | Hentriacontane  | 0.10          | C24H44            | 436.83           |
| 15.21                | 8-Heptadecane   | 0.23          | C17H34            | 238.45           |
| 15.44                | Heptadecane     | 0.06          | C17H36            | 240.46           |
| 15.70                | Phenol, 2-(1-phenylethyl)- | 0.19 | C14H26O | 198.26 |
| 16.46                | 1-Octadecane    | 0.47          | C18H36            | 252.48           |
| 16.53                | Octadecane      | 0.06          | C18H38            | 254.49           |
| 16.91                | Bicyclo[3.1.1]heptanes, 2,6,6-trimethyl- (1.alpha.,2.beta.,5.alpha) | 0.32 | C10H18 | 138.24 |
| 16.97                | 2-Undecanone, 6,10-dimethy- | 0.13 | C13H26O | 198.34 |
| 17.18                | Dibutyl phthalate | 0.30   | C16H22O4         | 278.34           |
| 17.36                | Phytol, acetate | 0.11          | C22H42O2         | 338.56           |
| 17.66                | Phthalic acid, butyl isohexyl ester | 0.45 | C18H36O4 | 306.39 |
| 17.84                | Phthalic acid, 2-ethylhexyl pentyl ester | 0.09 | C21H34O4 | 348.47 |
| 18.14                | 1,2-Benzenedicarboxylic acid, butyl | 2.47 | C20H36O4 | 334.44 |
| 18.32                | Phthalic acid, butyl isohexyl ester | 1.12 | C18H35O4 | 306.39 |
| 18.51                | 5-Eicosene, (E)- | 0.66 | C20H40 | 280.53 |
| 18.56                | Dodecane, 1',1'-oxybis- | 0.10 | C24H50O | 354.65 |
| 19.89                | Silanetriamine,1-azido-N,N,N',N',N'',N''-hexamethyl- | 0.21 | C6H13N5Si | 202.33 |
| 20.36                | Behenic alcohol | 0.26          | C22H44O2         | 326.60           |
| 20.87                | 1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester | 0.27 | C20H36O4 | 334.44 |
| 21.70                | Methyl dehydroabietate | 0.10 | C17H32O2 | 314.46 |
| 22.08                | Octacosanol     | 0.12          | C28H56O2         | 410.75           |
| 22.24                | Phenol, 2,4-bis(1-phenylethyl)- | 0.25 | C20H29N2O3 | 479.56 |
| 22.37                | Phenol, 2,4-bis(1-phenylethyl)- | 0.49 | C20H29N2O3 | 479.56 |
| 22.80                | Bis(2-ethylhexyl) phthalate | 0.45 | C24H38O4 | 390.55 |
| 23.02                | Naphthalene, 6-chloro-1-nitro- | 0.13 | C16H9ClNO2 | 207.61 |
| 23.21                | Bis(2-ethylhexyl) phthalate | 0.42 | C24H38O4 | 390.55 |
| 26.89                | Benzo[h]quinoline, 2,4-dimethyl- | 0.22 | C15H13N | 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 phytoxins, etc (Chu, 2012). It was reported that lipids obstruct microbes by 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 | Control tubs with V. harveyi (cfu/ml) | Treatment tubs extract with V. harveyi (cfu/ml) | Tubs with extract and PL alone (cfu/ml) | Tubs with PL alone (cfu/ml) | Total plate count | V. harveyi | V. harveyi | Treatment tubs | Control tubs | Temperature (°C) | Salinity (PSU) | pH in control | pH in treatment |
|-----|-------------------------------------|-----------------------------------------------|---------------------------------------|----------------------------|-------------------|------------|------------|----------------|-------------|-----------------|---------------|--------------|----------------|
| 0   | 0.00                                | 0.00                                          | 0.00                                  | 0.00                       | 1.24×10^8        | 1.15×10^8 | 2.41×10^6 | 17.7±3         | 18.1±2      | 29.0±1.0        | 20.0±0.5      | 8.40±0.2     | 8.30±0.2       |
| 5th | 13.66±0.3                           | 40.96±0.2                                    | 2.39±0.1                             | 3.23±0.1                  | 2.42×10^6        | 2.38×10^4 | 3.40×10^5 | 60.9±4          | 63.6±3      | 29.5±1.0        | 20.0±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 | 1.44×10^5 | 121.1±4         | 127.5±5     | 29.0±1.0        | 20.0±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 | 1.45×10^5 | 156.3±5         | 157.5±5     | 30.0±1.0        | 20.0±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                | 1.29×10^5        | 3.40×10^5 | 2.66×10^4 | 201.5±9         | 197.9±7     | 30.0±1.0        | 21.0±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                | 9.40×10^5        | 5.40×10^5 | 1.80×10^4 | 236.9±8          | 240.1±9     | 31.0±1.0        | 21.0±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                | 8.20×10^5        | 4.10×10^5 | 1.74×10^4 | 269.3±9          | 266.5±8     | 30.0±1.0        | 21.0±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 exist, 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 constituents like 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.

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