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Inhibitory effect of marine algae collected from the East and West coast of India against luciferase and luminescence producing *Vibrio harveyi*

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This study explored the development of inhibitors from marine algae against luminescence disease causing *Vibrio harveyi*. Organic solvents such as ethyl acetate (EA) and methanol:chloroform (CM) were treated to extract compounds from marine algae such as *Skeletonema costatum*, *Ulva fasciata* and *Kappaphycus alvarezii*. Antagonistic effect of algae extracts was determined against marine *V. harveyi*. EA extract of *S. costatum* and *U. fasciata* at 300 µg showed maximum zone of inhibition (12.6 and 12.3 mm) against *V. harveyi*. Similarly, CM extract of *S. costatum* also showed maximum zone of inhibition (8.3 mm). When crude EA extract of *S. costatum* at 300 µg/ml was used for *V. harveyi*, the level of luciferase was reduced to three to seven counts per second (CPS) as compared to the control for 4 days as read by luminometer. 300 µg/ml of CM extract of *U. fasciata*, reduced the luciferase to 3 to 6 CPS. When EA extract of *S. costatum* at (300 µg/ml) was treated against *V. harveyi*, the luminescence was reduced 10 to 15 CPS. The CM extract from *U. fasciata* showed reduction in the luminescence production (7 to 13 CPS). Considering the cumulative effect of anti-luminescence and anti-*Vibrio* activities, 300 µg of crude extract prepared from *S. costatum* in both solvents were confirmed as inhibitory to *V. harveyi*. Significant difference ($p<0.05$) was observed among the levels of luciferase and luminescence in all the extracts but *S. costatum* showed less. This study confirms that marine algae extracts can be used to control luminescent disease causing *V. harveyi* among the shrimp grow-out system and larviculture.

Key words: Algae extracts, antagonism, *Vibrio harveyi*, anti-luciferase, anti-luminescence.

INTRODUCTION

Diseases produced by luminescent bacteria, are a major problem in the shrimp larviculture and to certain extend among the grow-out systems in India. Among the *Vibrios*, *V. harveyi* is causing luminescence disease on the post-larvae in shrimp hatcheries and grow-out farming (Austin and Zhang, 2006). Nevertheless, antibiotics took part for most role for disease control in aquaculture (Ruangpan, 1998). Due to inappropriate course of application, anti-biotics led to the development of resistance among the microbes present in the aquaculture system. Resistance may be transferred among human being by integration of animal, fish farms and to food chain (Dang et al., 2011). Different technologies have been familiarized to control luminescent bacteria in shrimp grow-out and larviculture systems. One such a novel technique reported to work against the luminescent bacteria was the “Green water culture system” (Tendencia and Pena, 2003).A green water culture system is an innovative technique wherein

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shrimps or fish are co-cultured with abundant microalgae such as *Chlorella*. Probiotic bacteria have been used extensively for controlling various fish diseases but high doses are indispensable and unable to maintain in many cases (Defoirdt et al., 2007).

Bacterial bio-luminescence is catalyzed by luciferase, which is a secondary metabolic process that can be detected and assayed (Nackerdien et al., 2008). Many marine algae are containing natural bioactive compounds that act as potent antimicrobial agents (Helena et al., 2011), which may act against the luminescence disease causing *V. harveyi.* A wide range of compounds, particularly terpenes, polyphenolic compounds and steroids have been reported from various marine algae (Blunt et al., 2006). Smith et al. (2010) described about oxylipins and polyunsaturated aldehydes synthesized by diatoms such as *Skeletonema costatum* and *Thalassiosira rotula* which antagonized against many human pathogens. The solvent based crude extract of *S. costatum* was inhibitory to *V. anguillarum* and human pathogenic bacteria (Shanmugapriya and Ramanathan, 2011). Different derivatives of diterpenoids extracted from *Ulva fasciata* exhibited antagonism against *V. parahaemolyticus* and *V. harveyi* (Chakraborty et al., 2010). The crude methanol extract of *U. fasciata* showed inhibition against *V. alginolyticus* (Priyadharshini et al., 2012). The *Sargassum ilicifolium* and *Kappaphycus alvarezii* collected from different coastal regions of Tamilnadu were found inhibitory to human and animal bacterial pathogens (Rebecca et al., 2012).

Though, many research works are carried out on the antagonistic potential of marine algae extract against marine pathogenic bacteria, but there are not much studies against luminescence and luciferase produced by marine *V. harveyi*. Therefore, the present study was undertaken to analyse the inhibitory potential of selected marine algae extracts against the luminescent disease causing *V. harveyi*.

MATERIALS AND METHODS

Isolation of bacterial strains

Marine water and soil samples were collected from coastal areas of Muttukadu and Kovalam, Chennai, India. The samples were pre-enriched in alkaline peptone water for 12 h and serially diluted with normal saline (0.85%) and surface spread on thiosulphate citrate bile-salt sucrose agar medium (TCBS) and seawater complex (SWC) agar medium. Luminescent colonies were observed after 20 h of incubation at 28°C in SWC agar. The isolates were confirmed by various biochemical tests such as arginine dihydrolase (-), lysine (+), ornithine decarboxylase (+), gelatinase (+), Voges proskauer (-) and D-glucosamine (-) (Abraham and Palaniappan, 2004). *V. harveyi* isolates were reconfirmed by detecting the presence of hemolysin (*vhh*) gene by polymerase chain reaction (PCR) method. Crude bacterial lysates of all *V. harveyi* isolates were used for PCR (Maiti et al., 2009). The primers used in PCR were as follows: forward primer: 5'-CTTCACGCTTGATGGCTACTG-3', and reverse primer: 5'-GTCACCCAATGCTACGACCT-3' and the final product of 235 bp was obtained from the *vhh* gene DNA sequences.

25 µl of reaction mixture was carried out for each isolates in PCR and it contains 2.5 µl of 10 x PCR buffer (100 mM Tris-HCl pH 9.0, 15 mM $MgCl_2$, 500 mM KCl, 0.1% (w/v) gelatin), 200 µM of concentrations of each dNTPs, 10 picomoles of each primer, 1.5 U of *Taq* polymerase, the 2 µl of sample DNA and 1.25 µl of dimethyl sulphoxide (DMSO). The thermocylcer (Applied Biosystems, USA) was used for the PCR with 30 reaction cycles of initial denaturation at 95°C for 5 min; 95°C for 1 min, 50°C for 1 min, 72°C for 1 min and a final extension at 72°C for 5 min. The PCR products were separated on a 1% agarose gel and stained with ethidium bromide (0.5 µg/ml) and photographed using a gel documentation system (BIO-RAD, USA) (Maiti et al., 2009). Reference strains of *V. alginolyticus* (MTCC4182) and *V. harveyi* (MTCC3438) were used as negative and positive control, respectively. The pathogenicity of *V. harveyi* isolates were determined by spotting cells in 3% blood agar and the zone formation as haemolysis around the cell spot was confirmed as pathogen.

The isolates of *V. harveyi* were stored in luria-bertani (LB) broth with sterile glycerol (15% v/v).

Preparation of algae extract

Micro alga *S. costatum* was collected from the back waters of Muttukadu (latitude 12.806°N; longitude 80.248°E), Chennai India. Mass culture was carried out in out-door tanks. The cells were collected (30 × 10^5 cells/ml), shadow dried then subjected under solvent extraction. The macro algae like *U. fasciata* (latitude 8.124° N; longitude 77.312° W) and *K. alvarezii* (latitude 8.121° N; longitude 77.318° W) were collected in the intertidal zone of Muttam, Kanyakumari District, Tamilnadu, India (Figure 1). The algae samples were washed in freshwater (1% $KMnO_4$) (w/v) to remove the epiphytes, sand and other extraneous matters then shadow dried. The dried algae was weighed, pulverised using mechanical grinder and used for extracting crude fatty acid extracts. The solvents ethyl acetate (EA) and chloroform:methanol (CM) (1:1) (v/v) called combined solvents was used for algae extraction called cold extraction carried out at 30°C (Das et al., 2005).

Each algae extracts were prepared by taking 1.0 g of shadow dried algae powder mixed with 10.0 ml of solvent and shaker incubated at 30°C at 50 rpm for 96 h. Later, the extracts were filtered using Whatman No. 1 filter paper and rotary evaporated (at 30°C) and stored in darkness at 4°C for further use. The final extract was dissolved in 5 mg/ml of 30% (v/v) DMSO and used for ascertaining antagonism against *V. harveyi*.

Estimation of minimum inhibitory concentrations (MIC)

The minimum inhibitory concentrations (MIC) for the three marine algae extracts were evaluated (Islam et al., 2008). Dilution methods were used to determine the MIC of algae extracts. In dilution tests, *V. harveyi* was tested for their ability to produce visible growth on a series of SWC agar plates. The lowest concentration of the algae extract which inhibited the visible growth of *V. harveyi* was known as the MIC. Various concentrations (5 to 50 µg) of algae extracts were examined. The plates were incubated at 28°C/24 h and 20 µl of *V. harveyi* (1.8 OD or 2.19 × 10^7 cfu/ml) was tested for MIC on SWC agar medium.

Antagonism of algae extract against *V. harveyi* through "well diffusion assay"

20 µl of *V. harveyi* (1.8 OD) was taken into Petri plates. 40 ml of LB agar medium was transferred into the plate. Two wells were punched

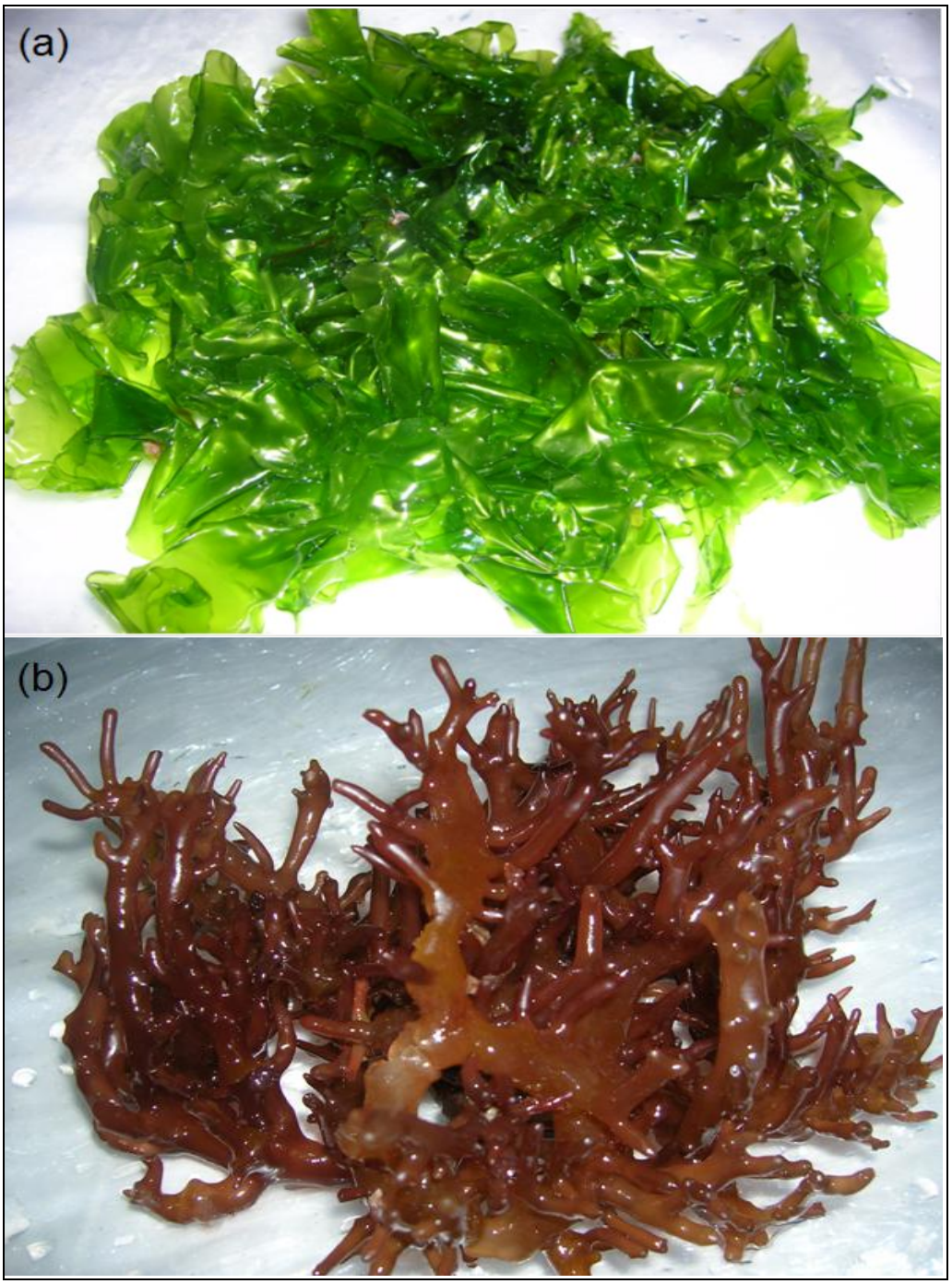

Figure 1. Marine macro algae collected from the west coast of India. (a) *Ulva fasciata* and (b) *Kappaphycus alvarezii.*

(6 mm) on the surface of the agar with sterile steel borer. The wells were sealed using 10 µl (0.8%) soft agar. 200 µl of algae extract was transferred into the well. The plates were incubated at 28°C for 24 h. The zone of inhibition was observed around the wells and measured in millimetre (mm). All the tests were performed in triplicates. 200 µl DMSO and 300 µg tetracycline were also placed in the wells as negative and positive control (Khairy and El-Kassas, 2010).

Effect of algae extracts against the growth, luciferase and luminescence produced by *V. harveyi*

Each algae extracts at 300 µg/ml was added in 100 ml LB broth. 500 µl of *V. harveyi* (1.8 OD) was inoculated in the LB broth and shaker incubated at 28°C/100 rpm for 4 days. Daily, 3 ml of spent culture was taken out and the growth of *V. harveyi* was measured at OD 600 nm for 4 days period. The *V. harveyi* cells were used to

test the luciferase production using the luciferase assay kit (Product No. LUC1, Technical Bulletin MB-260), Sigma, USA and read by luminometer (Victor^{TM} X3, Perkin Elmer, USA).

Cell lysate preparation

Cells from *V. harveyi* were harvested by centrifugation at 10,000 rpm for 5 min. The pellet was re-suspended in 333 µl of 1X cell lysis buffer (per ml of *V. harveyi*) and incubated for 10 min at 25°C. Pellets were centrifuged at 12,000 rpm for 1 min at 4°C. The supernatant was removed and stored in ice.

Luciferase assay

Luciferase substrate (lyophilized, luciferase assay substrate was suspended in luciferase assay buffer) as cell lysate containing luciferase has to be equilibrated to 25°C before use. 20 µl of the cell lysate was added to 100 µl of the luciferase substrate and mixed well. Reading was taken in 10 s for light emission after mixing and read by luminometer and expressed as counts per second (CPS, that is, photons per second). The light intensity was nearly constant for 20 s. LB broth medium and 1X lysis buffer was used as negative control for luciferase assay. For the estimation of luminescence, *V. harveyi* cells were harvested by centrifugation at 10,000 rpm/5 min and its spent culture medium was used (Kannappan et al., 2013).

Statistical analysis

Each algae extract was replicated thrice and expressed with mean value±standard deviations. Univariate analysis of variance following the statistical programme for the social sciences (SPSS, ver. 16.0) was used to assess the significance among the algae extracts treated against *V. harveyi* for ascertaining the luciferase and luminescence.

RESULTS

Identification and characterization *V. harveyi*

All the isolates were identified as the genus *Vibrio*, since they were Gram-negative rods, motile, oxidase-positive, fermented glucose and were sensitive to the vibriostatic agent O/129 and utilized D-mannitol as sole source of carbon. All isolates were confirmed as arginine dihydrolase-negative, lysine and ornithine decarboxylase-positive. Isolates Vh1, Vh2, Vh3 and Vh4 were identified as typical *V. harveyi* (citrate positive, negative for Voges-Proskauer; positive growth with 8% NaCl and negative with 0% NaCl). All isolates were produced bio-luminescence.

PCR for *vhh* gene

Among 14 isolates tested, only four isolates were amplified with the expected PCR fragment size of 235 bp using the *vhh* gene indicating the presence of hemolysin. Confirmed *V. harveyi* isolates were named as Vh1, Vh2, Vh3 and Vh4. Vh1 was used in the study.

MIC of algae extracts

The EA treated extract of *S. costatum* (25 µg) showed MIC and *U. fasciata* at 30 and 40 µg for *K. alvarezii*, respectively. The combined solvent treatment showed at 30 µg for *S. costatum*, at 50 µg for *U. fasciata* and 40 µg for *K. alvarezii*, respectively.

Antagonism of algae extracts against *V. harveyi*

The EA extract of *S. costatum* at 300 µg gave highest zone of inhibition (12.6 mm) against the growth of *V. harveyi* but at 200 µg showed 6.6 mm. The combined extract of CM treated *S. costatum* gave 8.3 mm zone of inhibition against *V. harveyi* (300 µg). EA extract of *U. fasciata* at 300 µg gave highest inhibitory zone (12.3 mm) against *V. harveyi* but at 200 µg, produced 8.3 mm. Combined extract of CM treated *U. fasciata*, at 300 µg showed less inhibition (6.6 mm). EA extract of *K. alvarezii* at 300 µg gave 8.6 mm inhibition against *V. harveyi* but the same extract at 200 µg showed 4.6 mm inhibition. Combined extract of CM treated *K. alvarezii* gave 5.6 mm inhibition on *V. harveyi* (300 µg). Compare to all the three EA treated marine algae extracts, *S. costatum* at 300 µg gave highest inhibition (12.6 mm) against *V. harveyi* followed by *U. fasciata* (12.3 mm). The lowest inhibitory zone was showed by *K. alvarezii* (8.6 mm). Among the three marine algae extracts, EA treated micro alga gave highest inhibition zone against *V. harveyi* than macro algae. Compared to all the three marine algae extracts treated in combination (CM), the micro alga *S. costatum* gave highest inhibitory zone (8.3 mm) followed by identical inhibitions by *U. fasciata* (5.6 mm) and *K. alvarezii* (5.6 mm).

Among the three marine algae tried, micro alga gave highest inhibition (8.3 mm) than macro algae (Table 1). DMSO was used to dissolve crude algae extracts and also used as the control.

Effect of algae extracts against the growth of *V. harveyi* in LB medium

The EA extract of *S. costatum* showed growth (OD) difference on *V. harveyi* that is, 0.223 on 1st day when compared to the control (1.937). On 2nd day, the OD reduced to 0.251. But on the 3rd day, more difference was observed on the growth (OD 0.375). EA extract of *U. fasciata* showed growth difference on *V. harveyi* as 0.468 OD on the 1st day, 0.521 on the 2nd day and 0.503 OD on the 4th day; but EA extract of *K. alvarezii* showed OD differences as 0.137 on the 1st day, 0.184 on 2nd day, 0.178 on 3rd day and on 4th day 0.132, respectively. CM extract of *S. costatum* showed the growth difference (OD) of *V. harveyi* as 0.156 in the 1st day, 0.209 in the 2nd day, 0.286 in the 3rd day and 0.263 in the 4th days; but CM extract of *U. fasciata* showed growth differences as 0.343

Table 1. Antagonism of marine algae extracts against *V. harveyi* through "Agar well diffusion Assay".

Algae species	Test bacteria	Zone of inhibition (mm)					
		Ethyl acetate extract (µg)			Chloroform: Methanol (1:1) extract (µg)		
		100	200	300	100	200	300
S. costatum	*V. harveyi*	3.3 ± 0.1	6.6 ± 0.2	12.6 ± 0.5	2.6 ± 0.1	4.6 ± 0.1	8.3 ± 0.4
U. fasciata	*V. harveyi*	3.3 ± 0.1	8.3 ± 0.3	12.3 ± 0.5	2.3 ± 0.1	3.6 ± 0.1	5.6 ± 0.2
K. alvarezii	*V. harveyi*	2.3 ± 0.1	4.6 ± 0.1	8.6 ± 0.4	2.6 ± 0.1	3.3 ± 0.1	5.6 ± 0.2

Zone of inhibition (mm), around the agar well, mean value of three replicates with SD (±).

in 1st day, 0.376 in 2nd day, 0.353 in 3rd day and 0.402 in 4th day as compared to control. Although, CM extract of *K. alvarezii* showed growth differences as 0.088 in the 1st day, 0.132 in 2nd day, 0.124 in 3rd day and 0.100 in 4th day as compared to control (Figures 2 and 3).

Effect of treatment of crude algae extracts against the changes of luciferase

For the crude EA extract of *S. costatum* when treated against *V. harveyi*, the production on luciferase was reduced to 8.0, 6.7, 3.3 and 7.0 CPS for four days as compare to control. The reduction of luciferase was maximum on the 1st, 2nd and 4th day of treatment. But on the 3rd day a minimum reduction on luciferase was observed (3.3 CPS). EA extract of macro algae *U. fasciata*, when treated against *V. harveyi*, luciferase production was reduced to 5.7, 6.7, 3.3 and 7.0 CPS for four days as compared to the control. The reduction on luciferase was maximum on the 4th day followed by 2nd and 1st day treatments but on the 3rd day a minimum reduction on luciferase was observed (3.3 CPS). EA extract of macro algae, *K. alvarezii* when treated against *V. harveyi*, the level of luciferase was reduced to 7.0, 4.7, 2.0 and 4.0 CPS for four days as compare to control. The reduction of luciferase was observed maximum on the 1st day followed by 2nd and 4th day, but on the 3rd day, a minimum reduction on luciferase was observed (2 CPS).

When chlorofom and methanol extract of micro alga *S. costatum*, was treated against *V. harveyi*, the luciferase level was reduced to 7.7, 5.7, 2.0 and 7.0 CPS for four days as compared to the control. The reduction of luciferase was observed maximum on the 1st day followed by 2nd and 4th day of treatment but on the 3rd day, a minimum reduction on luciferase was observed (2.0 CPS). When chlorofom and methanol extract of macro alga *U. fasciata* was treated against *V. harveyi*, the level of luciferase was reduced to 9.0, 7.0, 3.0 and 9.3 CPS for four days. The reduction of luciferase was observed maximum on the 4th day followed by 1st and 2nd day of treatment but on the 3rd day, a minimum reduction on luciferase was observed (3.0 CPS). When chlorofom and methanol extract of macro alga *K. alvarezii*, was treated against *V. harveyi*, the level of luciferase was reduced to 5.0, 4.7, 1.7 and 5.3 CPS for four days. The maximum reduction on luciferase was observed on the 4th day followed by 1st and 2nd day of treatment but on the 3rd day a minimum reduction on luciferase was observed (1.7 CPS).

When we compared the EA extract of *S. costatum* with CM extract of *S. costatum*, EA extract of *S. costatum* showed maximum reduction in luciferase for all the three days, except on 4th day with the variations on the luciferase level as 0.3, 1.0, 1.3 CPS, respectively. CM extract of macro alga, *U. fasciata* showed the maximum reduction on luciferase for all the three days except on 3rd day. The differences among the luciferase level were 3.3, 0.3, 0.3 and 2.3, respectively as compared to EA extract of *U. fasciata.* EA extract of macro alga *K. alvarezii* showed the maximum difference on the luciferase reduction (2.0) on the 1st day as compared to CM extract of *K. alvarezii.* At the same time, EA extract of macro alga *K. alvarezii* also showed reduction on luciferase (1.3 CPS) during the 4th day as compared with CM extract of *K. alvarezii* (Figure 2a to c).

Effect of treatment of crude algae extract against the changes of Luminescence

EA extract of micro alga, *S. costatum*, when treated with *V. harveyi*, the production of luminescence was reduced to 10.0, 11.0, 20.0, 25.0 CPS for four days as compared to the control (39.6, 50.3, 59.3 and 63.6). The maximum reduction on luminescence was reported during the 4th day (25 CPS) and minimum reduction was found during the 1st day (10.0 CPS). When EA extract of macro alga of *U. fasciata* was treated with *V. harveyi*, the production on luminescence was reduced to 7.3, 17.7, 13.0 and 17.0 CPS for the four days. The maximum reduction on luminescence was reported during the 2nd day (17.7 CPS) and minimum reduction was found during the 1st day (7.3 CPS).

When EA extract of macro alga of *K. alvarezii* was treated with *V. harveyi*, the production of luminescence was reduced to 6.0, 7.0, 9.7 and 16.0 CPS for the four days period as compared to the control. The maximum reduction on luminescence was reported during the 4th day (16.0 CPS) and minimum reduction was found during the 1st day (6.0 CPS). When CM extract of micro alga of

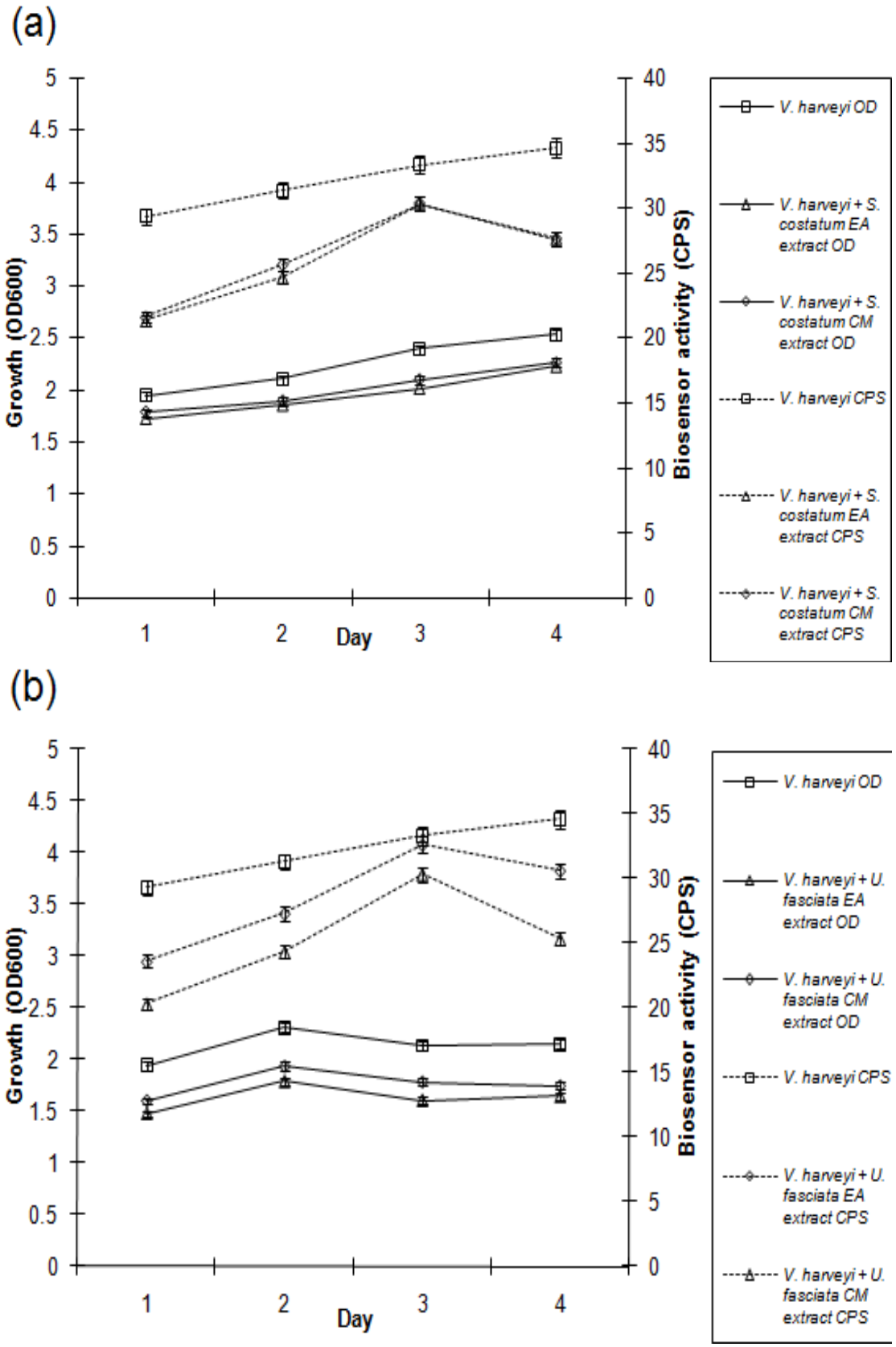

Figure 2. Effect of ethyl acetate and chloroform:methanol based algae extract of (a) *S. costatum*, (b) *U. fasciata* and (c) *K. alvarezii* against the luciferase produced by *V. harveyi.*

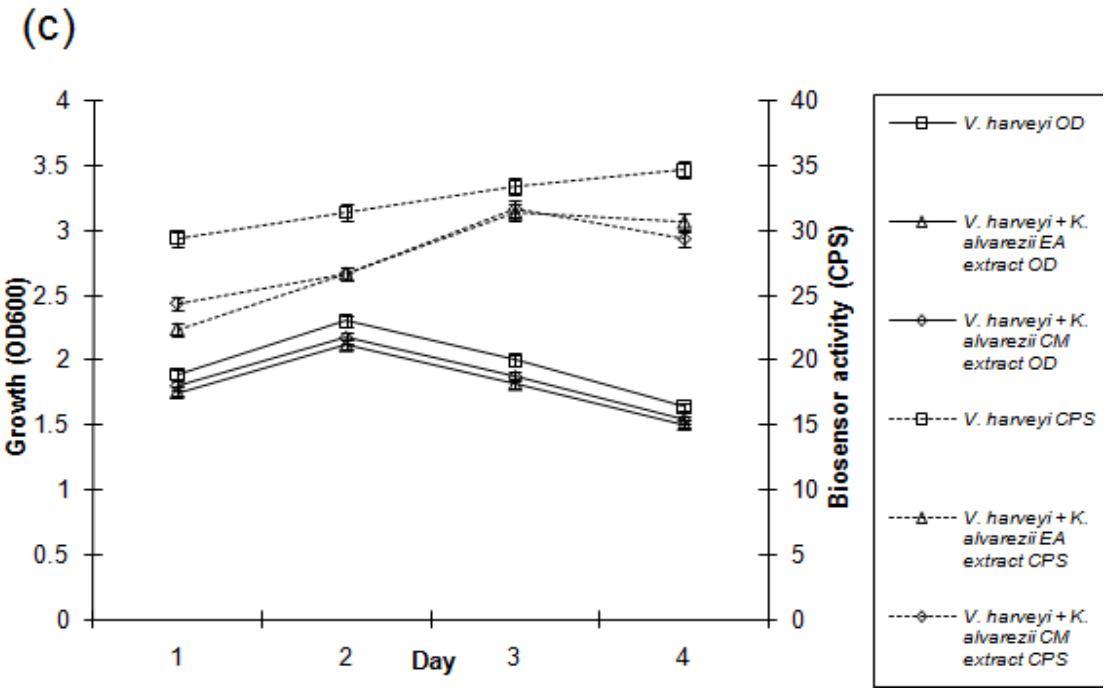

Figure 2. Contd.

S. costatum was treated against *V. harveyi*, the production of luminescence was reduced to 7.3, 7.7, 9.0, 12.3 CPS for four days period as compared to the control (39.6, 50.3, 59.3 and 63.6). The maximum reduction on luminescence was reported on the 4th day (12.3 CPS) and minimum reduction was found on the 1st day (7.3 CPS).

When CM treated extract of macro alga *U. fasciata* was treated with *V. harveyi*, the production of luminescence was reduced to 9.0, 10.0, 15.0 and 15.0 CPS for four days. The maximum reduction on luminescence was observed during the 3rd and 4th day (15.0 CPS) and minimum was treated with *V. harveyi*, the production of luminescence was reduced to 7.0, 12.0, 12.0 and 13.0 CPS for four days period. The maximum reduction on luminescence was reported during 4th day (13.0 CPS) and minimum reduction was found during the 1st day (7.0 CPS) (Figure 3a, b and c). Significant difference ($P<0.05$) was observed among all the algal extracts treated. There were less significant values noticed on the *S. costatum* extract treated with *V. harveyi* than for other algae extracts on the luciferase and luminescence reductions of this study.

DISCUSSION

From this study, it was apparent that even, single solvent treated marine algae at 300 µg exhibited maximum inhibition (8.6 to 12.6 mm) than combined solvents (5.6 to 8.3). Marine algae extract treated by single solvent even at 200 µg concentration exhibited less inhibition (4.6 to 8.3 mm) than those treated with combined solvents (3.3 to 4.6 mm). Marine algae have been considered as an enormous source of bioactive compounds and various kind of secondary metabolites used for innumerable biological activities (Cox et al., 2010). Many bio-active compounds such as cytostatic, antiviral, anti-helmintic, antifungal and antibacterial (Newman et al., 2003) have been detected in green, brown and red algae. Though, there are algae extracts reported as inhibitory to numerous aquatic and human pathogens, the study on antagonism of algae extracts against luciferase and luminescence producing *V. harveyi* is scarce, hence, this work was not much comparable with other research findings. The growth reduction on *V. harveyi* was noticed maximum on the 3rd day (0.532) by the EA extract of *U. fasciata*. Out of three EA treated micro and macro algae extracts against *V. harveyi*, maximum reductions were showed by macro alga *U. fasciata* followed by *S. costatum*. Out of three CM treated micro and macro algae extracts against *V. harveyi*, maximum reduction was showed by *U. fasciata* followed by *S. costatum.* The reduction on luminescence was varied with the days of treatment.

EA extract of *S. costatum* and *K. alvarezii* showed maximum luminescence reduction on the 4th day, whereas, EA extract of *U. fasciata* showed maximum luminescence reduction on 2nd day. It has also been proved that ethanol based extract of *S. costatum* showed

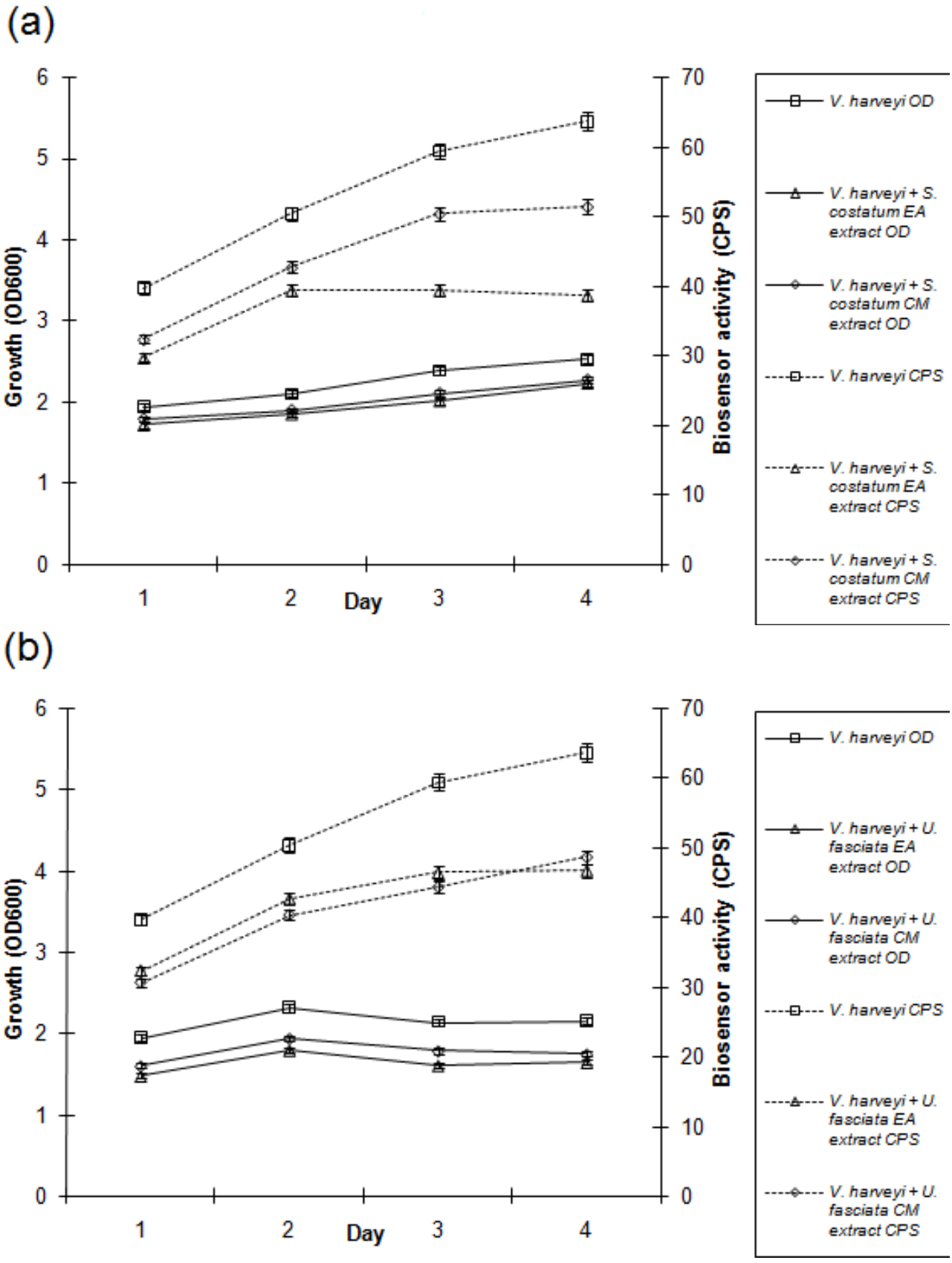

Figure 3. Effect of ethyl acetate and chloroform:methanol based algae extract of (a) *S. costatum*, (b) *U. fasciata* and (c) *K. alvarezii* against the luminescence produced by *V. harveyi.*

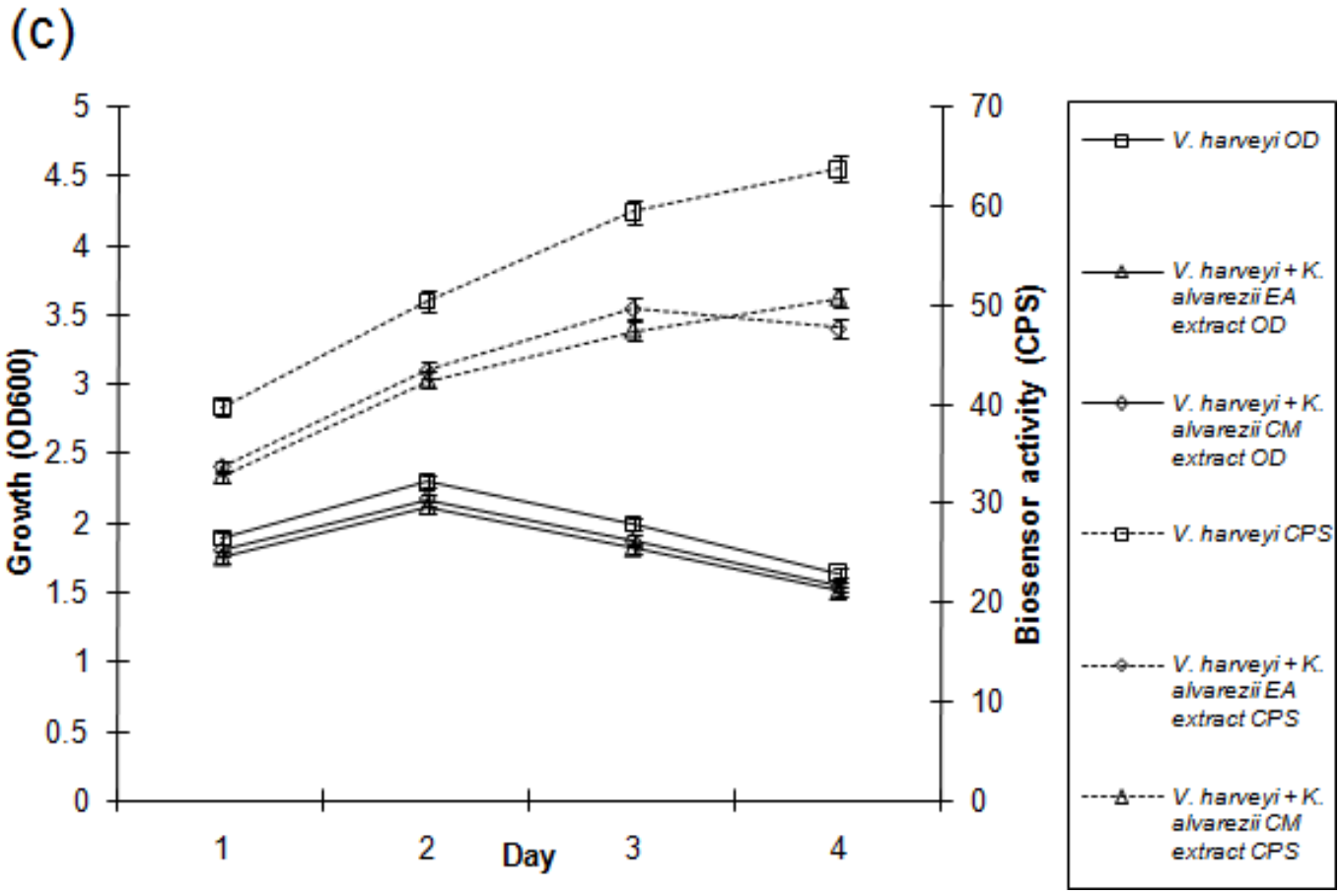

Figure 3. Contd.

zone of inhibition (15 mm) against several *Vibrio* species (Naviner et al., 1999). Shanmugapriya and Ramanathan (2011) reported that the diethyl ether extract of *S. costatum* at 100 μg/ml was inhibitory (20 mm) to various human pathogenic bacteria like *Klebsiella pneumoniae, Pseudomonas aeruginosa*, *E. coli*, *Staphylococcus aureus*, *Proteus vulgaris* and *Salmonella typhi.* Here, all the three EA treated extracts of marine algae showed reductions on luminescence in the increasing trend correspond to their incubation time. The minimum reduction on luminescence produced by *V. harveyi* also varied from all the three EA treated extracts of marine algae. Selvin and Lipton (2004) studied about the antibacterial activity of *U. fasciata* and *H. musciformis* extracts against *V. harveyi*, *V. fischeri* and *V. alginolyticus.* Solvents treated green alga, *Ulva reticulata* showed maximum zone of inhibition against *Vibrio*s 4, 8 and 4 mm, respectively (Karthikaidevi et al., 2009). *K. alvarezii* treated with chloroform, ethanol and methanol showed inhibition (30 mm) against human pathogens (Rebecca et al., 2012).

The luciferase is produced within the cells and the luminescence takes place extracellular. In the present study, the extra cellular luminescence activity was measured from the spent culture of *V. harveyi* treatment against algae extract. For the crude EA extract (300 μg/ml) of *S. costatum*, when treated with *V. harveyi*, luminescence activity was reduced from 10 to 25 CPS as compared to the control. But the crude CM extract from *U. fasciata* showed reduction of luminescence activity from 10 to 15 CPS. It has been reported that the fresh water algae such as *Chlamydomonas reinhardtii* and *Chlorella* sp stimulated quorum sensing dependent luminescence on *V. harveyi* (Teplitski et al., 2004). Macro alga *Ulva was* used for wastewater treatment and showed significant reduction on coliforms bacteria (Correa et al., 2010). The other evidence shows that the ethanolic extract of 24 Indian medicinal plants were tested for antiquorum sensing activity using *Chromobacterium violaceum* as reporter strain (Zahin et al., 2010). The results of this study show that crude extracts of marine algae inhibited the growth of *V. harveyi.* It was evident that the algae extracts, reduced the level of cellular luciferase and extracellular luminescence produced by *V. harveyi*. Based on the results, 300 μg of *S. costatum* extract was able to give better anta-

gonism against the growth of *V. harveyi*. Considering the cumulative effect of luciferase and luminescence activities both in the cellular and extra cellular level, along with the growth of *V. harveyi*, 300 μg crude extract of *S. costatum* in both solvents have proved to be the effective products for controlling *V. harveyi.* Further, studies on the active compounds involved from this crude extract and their mechanistic action is going on. These crude algae fatty acid extracts may be used to control luminescence causing *V. harveyi* in the aquaculture system.

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