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Docking Studies on Biomolecules from Marine Microalga *Skeletonema costatum* Against Hemolysin Protein of Bioluminescence Disease-Causing *Vibrio harveyi*

Krishnamoorthy Sivakumar[1] · Sudalayandi Kannappan[2] · Balakrishnan Vijayakumar[3,4,5]

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Abstract
Grow-out and hatchery units of shrimps are being impacted by disease-causing bacterial pathogens and predominantly marine *Vibrios*. The use of chemicals for governing bacterial pathogens in the aquaculture practices is developing resistance to bacteria. Henceforth, the application of bio-therapeutic agents from marine resources for controlling pathogens is most vital to be considered. Molecular docking is computer-assisted drug design tool to detect and counteract for drug–receptor interaction for known target protein of diseases. Therefore, an effort was made with the extract of the marine micro alga *Skeletonema costatum* against hemolysin protein of pathogenic bacteria *Vibrio harveyi*. The extract of *S. costatum* was tested against growth and virulence produced by *V. harveyi* during larviculture of *Penaeus monodon*. The extract was analyzed for phyto-constituents through GC–MS and used them as ligand molecule in docking. *S. costatum* extract at 200 µg mL^{-1} was found to decrease 35.20% of cumulative percentage mortality (CPM) in postlarvae of *P. monodon* against *V. harveyi* infections. The biomolecule Docasane, an alkane from the extract of *S. costatum*, exposed highest binding interaction than other compounds during docking analysis. The level of significance ($P < 0.05$) was found in CPM, growth, and virulence factors of *V. harveyi* studies. Thus, the present finding predicts that extract of *S. costatum* containing biomolecules can be recommended for use in the shrimp culture-based grow-out and hatchery units for eliminating bioluminescent *V. harveyi*.

Introduction

Marine *Vibrios* cause major bacterial diseases problems in the aquaculture systems. The bioluminescence disease-causing *Vibrio harveyi*, which is found in seawater, marine sediments, guts of sea foods, on the surface of decomposing fish, etc. [1]. *V. harveyi* causes infection in whiteleg shrimp, *Litopenaeus vannamei*, and significantly alters amino acid and carbohydrate metabolism with extensive changes in the hepatopancreas [2]. *V. harveyi* produces various virulence factors, such as bioluminescence, phospholipase, hemolysin, proteases, exopolysaccharides, crude extracellular protein (bacteriocin), and cell surface hydrophobicity [3]. Hemolysin is the most commonly distributed exotoxin among the pathogenic *Vibrios*, and there is much epidemiological and experimental evidence to suggest involvement in disease pathogenesis [4]. *V. harveyi* is known to exhibit multidrug resistance [4] against various chemical treatments used in aquaculture.

Among the pathogenic *Vibrios*, hemolysins are known for their capability to lyse erythrocytes and hemolysin toxins involve two modes of action, including cell pore-forming

✉ Krishnamoorthy Sivakumar
ksivafbt@gmail.com; sivakumark@tanuvas.ac.in

1 ICAR – Krishi Vigyan Kendra, Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Kattupakkam, Chennai, Tamil Nadu 603 203, India

2 Crustacean Culture Division, ICAR – Central Institute of Brackishwater Aquaculture (CIBA), Chennai, Tamil Nadu 600 028, India

3 Centre of Advance Study in Crystallography and Biophysics, University of Madras, Chennai, Tamil Nadu 600 025, India

4 McArdle Laboratory for Cancer Research, Department of Oncology, School of Medicine and Public Health, University of Wisconsin at Madison, Madison, WI 53705, USA

5 Sao Carlos Institute of Physics (IFSC), University of Sao Paulo, Av. Joao Dagnone, 1100 – Jardim Santa Angelina, Sao Carlos 13563-120, Brazil

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protein (Fig. S1) and phospholipase enzyme activity [4]. Further, *V. harveyi* hemolysin (*vhh*) protein was reported to produce cytotoxin in the gills of Flounder fish during tissue culture and revealed strong pathogenicity when administered [5]. The virulent isolates of *V. harveyi* were found to express the *vhh* gene during infection with brine shrimp (*Artemia* sp.) larvae, while non-virulent isolates revealed lower expression of the *vhh* gene [4]. Among the shrimp grow-out and larviculture practices of *L. vannamei* and *Penaeus monodon*, the luminescent *Vibrios* caused mass mortalities [6]. Several evidences also acclaimed that post-larval stages of shrimps are more vulnerable to *V. harveyi* infection than the juvenile and adults and cause mass mortalities up to 80–100% [6]. The development of antimicrobial resistance has increased in aquaculture; correspondingly there is a need for developing new antimicrobial agents that will be more selective, potent, and less toxic as compared to the existing drugs in clinical treatment.

Many herbal plant based diets were fed to the postlarvae of *P. monodon* and tested against the infections caused by *V. harveyi*. The results showed a 75% improvement in survival with improved weight gains [7]. The fucoidan obtained from brown algae (*Undaria pinnatifida*) was administered through a feed, during *P. monodon* larviculture and found reduction in the mortalities caused by *V. harveyi* [8]. The extract from brown algae (*Endarachne binghamiae*) when administered through *Artemia* nauplii, against the infection caused by *Vibrio alginolyticus* [9], which has increased survival of postlarvae of *L. vannamei*. The crude garlic extract, when fed through feed with the larviculture system of *P. monodon*, a significant reduction on the growth and mortality of postlarvae against *V. harveyi* infections [10] also reductions in the virulence factors, such as luciferase and bioluminescence. When the crude extract of marine macro alga *Kappaphycus alvarezii*, tested against luminescence disease-causing *V. harveyi* and its virulence factors during *P. monodon* larviculture [6], antagonism was found. Ethyl acetate and combined solvent-based various marine algal crude extract, when tested against the luciferase and luminescence producing *V. harveyi*, the growth of *V. harveyi* was reduced with virulence factors. But the better level of inhibition on growth was observed in ethyl acetate [11] based solvent extraction. Enhanced extraction of lipids/compounds from microalgae with solvents like methanol and ethyl acetate for biodiesel production was studies individually [12]. But much of biodiesel/lipids were noticed ethyl acetate-based solvent extraction methods. Further, seasonal-based evaluation of phlorotannin-enriched extracts from macro algae, *Fucus spiralis*, was calculated using ethanol and ethyl acetate extractions separately and observed better level of inhibitions was found only in ethyl acetate solvent extraction method [13]. In the crude compounds present in the algae extract, which of the compounds inhibits the microbes has to be evaluated [14]. A broad range of compounds, regulations, and risk assessment methodologies including animal studies are covered, in order to identify mixtures of concern, gaps in the regulatory framework, and data requirement are to be carried out. Also the current and potential future use of novel tools (Adverse outcome pathways, in silico tools, toxicokinetic modeling) in the risk assessment of combined effects were studied [15]. Under this situation molecular docking (in silico analysis) is one of the best tool and cost effective to identify the effect of individual molecule (ligand) playing with target diseases without scarifying the animals and before to isolate or bulk production, etc. [15].

In recent years molecular docking has played a crucial role in in silico drug development. The important advantage of the tools is that they require less investment in resources and time in comparison to the in vivo lab studies [15]. Molecular docking is usually done between a small molecule and a target macromolecule, which is often referred to a ligand–protein docking [16]. The ligand–protein docking aims to predict the predominant binding mode(s) of a ligand with a protein of known three-dimensional (3D) complex structure. Due to the lack of an experimentally established crystal structure of a given protein, homology modeling is the best alternative to construct a reasonable (3D) model of the target [17]. Also generating binding energies in docking studies, the position of the ligand in the enzyme-binding site can be visualized [18]. In such a way, the marine diatom, *Skeletonema costatum* present in the coastal marine habitats, is being extensively used to feed larvae of crustaceans. *S. costatum* extract has been reported to contain various bioactive compounds and its antibacterial activity has been reported against fin fish and shell fish bacterial pathogens [19]. The compounds oxylipins and polyunsaturated aldehydes synthesized by *S. costatum* and *Thalassiosira rotula*, which antagonized various human pathogens [20]. The crude extract of *S. costatum* was found to be inhibitory against marine pathogen *Vibrio anguillarum* and human pathogens [21]. Henceforth, this study was proposed to do docking study on biomolecules extracted from micro alga *S. costatum* against hemolysin protein of biomolecules disease-causing *V. harveyi* and developing bio-agents to control *V. harveyi* in the aquaculture practices.

Materials and Methods

Isolation of *V. harveyi*

Vibrio harveyi bacterial strains were isolated from the infected *P. monodon* larviculture tanks (Indian Council of Agricultural Research (ICAR)-CIBA, Muttukadu Experimental Station, Chennai, India). The water samples collected from *P. monodon* larviculture tanks were enriched with

alkaline peptone broth (pH − 8.4 ± 0.2) (Hi-Media, India; Purity 99.5%) and shaker incubated at 28 °C for 24 h. The inoculums was serially diluted with normal saline (0.85%) and surface spread on Thiosulphate citrate bile-salt sucrose agar medium (TCBS) (Hi-Media, India; Purity 99.5%) and seawater complex (SWC) agar (Hi-Media, India; Purity 99.5%). Luminescent colonies were observed after 24 h of incubation in SWC and TCBS agar. After the 16–24 h, only luminous colonies were selected and streaked in Tryptone Soya Agar (TSA) (Hi-Media, India; Purity 99.5%) slants supplemented with 2% NaCl (Hi-Media, India; Purity 99.9%). The agar plates were also incubated as detailed above. Further, they were confirmed using phenotypic and genotypic characterization. *Vibrio harveyi* isolates ($n = 50$) were characterized by [6] using standard morphological, physiological, and biochemical tests. *Vibrio harveyi* isolates were streaked on TSA plates and incubated at 28 °C for 24 h. The colonies were initially characterized by Gram staining, oxidase, catalase, and oxidation/fermentation tests. *V. harveyi* isolates were further subjected to various tests such as motility, methyl red (MR)/ Voges–Proskauer (VP), salt and temperature tolerance, indole, O/129 sensitivity, citrate, urease, nitrate reduction, amino acid decarboxylase, pigment production, bioluminescence, amylase production, gelatin hydrolysis, lipase hydrolysis, hemolysis, and sugar fermentation for phenotypic characterization. References strains of *V. alginolyticus* (MTCC—4182) and *V. harveyi* (MTCC—3438) were obtained from MTCC and used for this study as negative and positive control, respectively. The confirmed isolates were maintained in Luria–Bertani (LB) broth (Hi-Media, India; Purity 99.5%) supplemented with 2% NaCl, 15% glycerol (SRL, India; Purity 99.5%), and stored at − 80 °C. *V. harveyi* isolates were reconfirmed by detecting the presence of hemolysin (*vhh*) gene by polymerase chain reaction (PCR) method as genotypic characterization [22]. Crude bacterial lysates of all *V. harveyi* isolates were used for PCR. The primers used in PCR were as follows: forward primer: 5′-CTTCACGCTTGATGGCTA CTG-3′ and reverse primer: 5′-GTCACCCAATGCTACGAC CT-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 10X 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 pmol of each primer, 1.5 U of Taq polymerase, 2 µL of sample DNA, and 1.25 µl of Dimethyl sulphoxide (DMSO) (Hi-Media, India; Purity 99.5%). 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^{-1}) and photographed using a gel documentation system (Bio-Rad, USA). The reference strains of *V. alginolyticus* (MTCC—4182) and *V. harveyi* (MTCC—3438) were used as negative and positive control, respectively. The pathogenicity of *V. harveyi* isolates were determined/further reconfirmed [6] by spotting cells in 3% blood agar (Hi-Media, India; Purity 99.5%) and the zone formation as hemolysis around the cell spot was confirmed as pathogen.

Collection of *S. costatum* and Preparation of Crude Extracts

The marine diatom *S. costatum* was collected from the backwaters of Muttukadu (Latitude 12.806° N; Longitude 80.248° E), Chennai, India using 10-µm plankton net. The cells of *S. costatum* were identified as cylindrical with a long tubular structure under the microscope. *S. costatum* cells (~ 30×10^5 cells mL^{-1}) were then mass cultured in outdoor FRP tanks (5000 L) using Guillard's algal medium (F/2) at 28 °C/@ 6000 Lux [23] with aeration. The cells were harvested and then shade dried, later stored at 4 °C. One gram of *S. costatum* powder was mixed with 10 mL of ethyl acetate (SRL, India; Purity 99.5%) and shaker incubated at 30 °C at 50 rpm/96 h and extracted for compounds called as "cold extraction method" [6]. Subsequently, the extracts were filtered by Whatman filter paper (No.1), then rotary evaporated at 30 °C for 3 h, and later stored in darkness at 4 °C. The resultant extract was liquefied with 5 mg/mL of 30% (v/v) DMSO.

Preparation of Inhibitors Data for Molecular Docking

The crude ethyl acetate extract of *S. costatum*, identified through Gas Chromatography and Mass Spectrometry (GC–MS) analysis for phytochemical constituents was used as ligand molecules in docking. GC–MS analysis was performed using Agilent GC–MS-5975C (Agilent 5975C TAD Series GC/MSD System, USA) with the triple-axis detector equipped with an auto sampler. The GC column used was fused silica capillary column (length 30 m × diameter 0.25 mm × film thickness 0.25 µm) used 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; oven temperature was 70 °C/3 min, which rose to 250 °C @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 were recorded from 50 to 400 Dalton per second and scan speed was 2000. Mass start time was at 5 min and end time at 35 min. Peak identification

of crude *S. costatum* extract was performed by comparison with retention times of standards/homologous series (Sigma-Aldrich, USA; Purity 99.5%; Product codes—402915, 8.22046, 175366, P5177, M3378, CRM46903, 1.09754, M3164) and also the mass spectra obtained were compared with those available in the NIST 20 libraries (2020) by an acceptance criterion of a match above a critical factor of 80% [6]. PUBCHEM (http://pubchem.ncbi.nlm.nih.gov) is a 3D structural database, organized as three linked database within the NCBI's Entrez information retrieval system. The 3D GC–MS compounds (inhibitors) were downloaded from this database. These 3D structures were converted to PDB (Protein Data Bank) format using PyMOL (0.99rc6) software [24] and these PDB files were used for docking.

Homology Modeling of Hemolysin Protein of *V. harveyi*

The 3D structure of the hemolysin protein of *V. harveyi* was not available (as of June 2018) in the Protein Data Bank (PDB; www.pdb.org). The primary sequence of hemolysin protein of *V. harveyi* was obtained from the Protein sequence ID is AAG25957.1 from NCBI. However, there was no suitable template available for hemolysin of *V. harveyi*. Therefore, the sequence was used to build the 3D model of protein structure using Phyre2 (Protein Homology/AnalogYRecognition Engine, Version 2.0) [25]. Hence, PDB id of 3KVN_A (1–311) template has been selected and the modeled structure was validated in the Phyre server for structural similarity analysis against the PDB database [25]. Phi/Psi dihedral angle for the predicted model was validated using Ramachandran plot from PROCHECK [25]. The modeled structure has 5.29% of amino acid residues in outliers of the Ramachandran plot.

Preparation of Hemolysin Protein and Actives Site Prediction

The structure modification was done in hemolysin protein for docking studies like the addition of hydrogen atoms, assigning correct bond orders, fixing the charges, and orientation of groups. Following this, optimization of the amino acid orientation of hydroxyl groups and amide groups of ASN, GLN, and HIS was carried out. All amino acid flips were assigned and H bonds were optimized. Non-hydrogen atoms were minimized until the average root mean square deviation reached a default value of 0.3 Å [26]. For docking, the active site (binding site) of hemolysin protein was determined by superimposing with esterase EstA protein from *Pseudomonas aeruginosa*. The active site residues SER153, HIS393, and ASP390 were considered as the catalytic reaction mechanism of the hemolysin protein of *V. harveyi* [27].

Docking Protocol

AutoDock 4.0 program was used to investigate the affinity of marine algal inhibitors at the binding pocket of hemolysin protein of *V. harveyi* through the implemented empirical free energy function and the Lamarckian Genetic Algorithm (LGA). While docking and extended PDBQT format of the PDB file was used as a coordinate file, which includes atomic partial charges. All the hydrogen atoms of the macromolecule (protein) were added using the AutoDock Tools software (Version 1.5.2 revision 2) for preparation of the target protein hemolysin (unbound target), which is a necessary step calculating partial atomic charges. Kollman charges were calculated for each atom of a macro molecule using Auto Dock 4.0. During the docking, the grid dimensions were $60 \times 60 \times 60$ Å with points separated by 0.375 Å, and the grid center was set to 1.778, 36.282, and 81.809 for *X*, *Y*, and *Z*, respectively, which covered all the active site amino acids include important 3 amino acid residues (SER153, HIS393, and ASP390) in the considered active pockets. LGA was employed as the docking algorithm with 10 runs, 150 population sizes, 2,500,000 maximum numbers of energy evaluations, and 27,000 maximum numbers of generations. The best-performing compounds were ascertained by the computation of drug-likeness properties. The drug-likeness scores of the compounds were evaluated with the rules of Lipinski's rule. During the docking process, a maximum of 10 conformers or poses were considered for each compound. In the present study, the best interaction(s) pose was showed by protein and ligand molecule along with least binding energy exhibited (kcal/mol) that was considered as the highest inhibiting activity of the respective compound and compared to the control molecule Oxytetracyline dihydrate (OTC) (Hi-Media, India; Purity 99.9%). The binding energy of the individual compound was decided based on the amount of free energy required concerning the interaction(s) of ligand molecule with active site amino acid residues of target protein either by H-bond or hydrophobic interactions. Besides, other docking parameter results such as inhibition constant (mM) and intermolecular efficiency (kcal/mol) were also considered along with binding energy exhibited, since they are directly proportional to binding energy [25]. PyMOL and UCSF Chimera (Version 1.8.1) software [25] were used for graphical visualization, analyzing of hydrogen bond interactions, and producing quality images. Hydrophobic interactions were observed between protein and ligand using Chimera software. All the docking calculations were performed with Intel® Core™ i3-2310 M CPU@2.10 GHz of 32-bit operating system of Lenovo, with 2.00-GB RAM. AutoDock 4.0 was compiled and run under Microsoft Windows 7 operating system.

Evaluating Drug-Likeness of the Biomolecules

An online software tool of Lipinski filter (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp) was performed to evaluate the information about drug-likeness properties of bio-compounds with the help of Lipinski rule of five [25]. This rule helps in distinguishing between drug-like and non-drug-like molecules. It predicts high probability of success or failure due to drug-likeness for molecules complying with two or more of the following rules: (i) Molecular mass less than 500 Dalton, (ii) High lipophilicity (expressed as LogP less than 5), (iii) Less than 5 hydrogen bond donors, (iv) Less than 10 hydrogen bond acceptors, and (v) Molar refractivity should be between 40 and 130.

Testing of *S. costatum* Extract Against *V. harveyi* During *P. monodon* Larviculture

The plastic tubs were washed with 10 mgL^{-1} of (w/v) potassium permanganate ($KMnO_4$-Hi-Media, India; Purity 99.9%) solution for 10 min and filled with 20 L of low saline water (20 Practical Salinity Units, PSU). Disease-free postlarvae (PL 10 days old) of *P. monodon* procured from private shrimp hatchery located nearby Chennai, Tamil Nadu India. The postlarvae were acclimatized at 20 (PSU) for 5 days at 29 ± 1 °C with continuous aeration. One hundred numbers of postlarvae (Average body weight 17–18 mg) were stocked in each tub. The control tub was inoculated with *V. harveyi* (10 mL of 1.80 OD) alone. The treatment tub was inoculated with *V. harveyi* and 200 µg of crude extract per ml (2 g/10 L). The third tub was considered as another control where crude extract alone added at 200 µg mL^{-1} with PL. The 4th tub was additional control for PL with neither *V. harveyi* but OTC (at 200 µg mL^{-1}) was also added. The aeration was given in each tub and PL feed at 15% of body weight was given twice. The water quality parameters such as temperature, salinity, and pH were mentioned for 5 days. The mortality of PL was counted every day and represented as cumulative percentage mortality (CPM). No water exchange was given for all the tubs until 30 days. The water samples were collected from the tanks once in 5 days. No water exchange was given for all the tubs until 30 days, but the sterile seawater was added to compensate the evaporated water. The water samples were collected from the tanks once in 5 days. Then total heterotrophic bacteria, luminous *V. harveyi* counts were enumerated using TSA and *Vibrio harveyi* selective agar (VHSA) medium [6]. All the experimental tubs were covered on the top to avoid the possibility of any external contaminations. For each experiment, the triplicate was maintained and average values were presented with SD [28].

Effects of *S. costatum* Extract Against Growth and Virulence Factors of *V. harveyi* During *P. monodon* Larviculture

The effects of *S. costatum* extract against virulence factors produced by *V. harveyi* were evaluated with shrimp postlarvae for every 5 days. The bioluminescent *V. harveyi* was treated against *S. costatum* extract. Then the *V. harveyi* was identified by VHSA medium, later inoculated into LB broth and incubated at 28 °C/100 rpm/24 h. After 24 h, the spent culture of *V. harveyi* was analyzed for virulence factors, such as hemolytic and phospholipase activities, extra crude protein (Bacteriocin), luciferase, and bioluminescence, protease enzyme production, and the growth. Cell surface hydrophobicity was observed by the Salt aggregations test [16]. The results of the agar plate assay were evaluated and graded based on the hydrolysis of the medium around the inoculated colonies. The activity was coded by qualitative parameters, like non-existent (−), weak (+), moderate (++), high (+++), and very high (++++) [6].

Statistical Analysis

In the present study each test/trial was performed in triplicates in control and treatment. The mean value was expressed in standard deviation (SD). For statistical evaluation, the one-factor analysis of variance (ANOVA) was followed using SPSS ver.16.0 software and assessed the significance (P value) among the treatment and control. In the current study, the P value was considered at least $P < 0.05$ level only.

Results

Isolation and Characterization of *V. harveyi*

All the *Vibrio* isolates were identified and confirmed as *V. harveyi*, since they were Gram-negative rods, motile, oxidase-positive, fermented glucose, and utilized D-mannitol as the sole source of carbon [29]. Among the isolated that were tested which were amplified with the expected PCR fragment size of 235 bp (Fig. S2) using the *vhh* gene indicating the presence of hemolysin. The *V. harveyi* isolates identified were named as (*Vh1* to *Vh20*) and isolate, *Vh1* was used in the present study (Table S1).

Effects of Various Biomolecules Obtained from *S. costatum* Extract Against Hemolysin Protein of *V. harveyi*

The biomolecules were known by GC–MS analysis from extract of *S. costatum* is shown in Table 1. GC–MS

Table 1 GC–MS profile of *S. costatum*

Retention time (min)	Compound name	Standards/homologous series (min)	Peak area (%)	Molecular formula	Molecular weight
9.40	Pentadecanoic acid, 13 methyl, methyl ester	9.40	18.94	$C_{17}H_{34}O_2$	270.45
10.30	16-Octadecanoic acid, methyl ester	10.30	48.05	$C_{19}H_{36}O_2$	296.49
10.41	3-Octadecanoic acid, methyl ester	10.41	11.22	$C_{19}H_{36}O_2$	296.49
11.31	Heptadecanoic acid, methyl ester	11.31	1.58	$C_{18}H_{36}O_2$	284.47
12.56	9-Octadecanoic acid, methyl ester	12.56	2.75	$C_{19}H_{36}O_2$	296.49
13.62	Tetradecanoic acid, methyl ester	13.62	3.54	$C_{15}H_{30}O_2$	242.40
14.88	*n*-hexadacanoic acid, methyl ester	14.88	4.64	$C_{17}H_{34}O_2$	270.45
18.45	Octadecanoic acid	18.45	2.57	$C_{18}H_{36}O_2$	284.48
19.64	Docasane	19.64	2.09	$C_{22}H_{46}$	310.60
22.85	1,2, benzenedicarboxylic acid	22.85	4.25	$C_8H_6O_4$	166.13

analysis has shown totally 10 compounds. Under molecular docking, there are 10 compounds (inhibitors) used for docking analysis against *V. harveyi* hemolysin protein and found interaction only by 9 compounds with important active site residues, i.e., exhibited H-bonding or hydrophobic interactions or both with 3 important active site residues (SER153, HIS393, and ASP390) including other residues of hemolysin protein of *V. harveyi*. The results of the interactions were found between hemolysin protein of *V. harveyi* and algal inhibitors with its docking scores obtained are shown in Table 2. The different model of hemolysin protein of *V. harveyi* alone and its best interaction pose was formed with OTC as depicted in 3D structure/view (Fig. 1 and Fig. S3). Besides, the 2D structure/view of the interacted molecules with hemolysin protein of *V. harveyi* are shown in Fig. 2. Based on the results of docking scores obtained from the compounds of *S. costatum* with *V. harveyi* hemolysin protein, the Docasane was exhibited highest inhibiting activity with docking scores like binding energy (− 4.28 kcal/mol), inhibition constant (0.733 mM), and intermolecular efficiency (− 0.19 kcal/mol) followed by Heptadecanoic acid, the methyl ester of binding energy (− 3.69 kcal/mol), inhibition constant (1.990 mM) and intermolecular efficiency (− 0.18 kcal/mol), Tetradecanoic acid, the methyl ester of binding energy (− 3.67 kcal/mol), inhibition constant (2.020 mM) and intermolecular efficiency (− 0.22 kcal/mol) and Pentadecanoic acid, 13 methyl, methyl ester of binding energy (− 3.58 kcal/mol), inhibition constant (2.36), and intermolecular efficiency (− 0.19), respectively, when compared to reference drug (Control molecule) OTC of binding energy (− 0.566 kcal/mol), inhibition constant (0.071 mM) and intermolecular efficiency (− 0.17 kcal/mol) in the present study in 3D structure/view including other molecules interacted with hemolysin protein (Fig. 3). Moreover, the screening of ligand molecules for predicting the drug-likeness of the compounds was performed based on the Lipinski rule of five. The Lipinski filter analysis revealed that all the 9 compounds of *S. costatum* possessed drug-likeness properties (Table 3).

Testing of *S. costatum* Extract Against *V. harveyi* During *P. monodon* Larviculture

When *S. costatum* extract tested against *V. harveyi* during *P. monodon* larviculture, it was found that CPM reduction was 35.20% when compared to control (76.30%) for 30 days. Three trials were maintained under larviculture as negative controls to distinguish if any effect of the extract on postlarvae (PL), another control for PL, and comparing algae extracts with control molecule OTC. However, it was observed that treatment did not affect the PL, which showed a lesser reduction on CPM, with extract, and PL (29.56%) and PL alone (28.39%) and PL challenged with OTC (46.80%). The weight of the PL was measured for control and treatment. There was not much weight difference was observed both in the treatment and control. On the final (30th day) sampling, the average weight of the PL was 279.8 mg and 277.4 mg for control and treatment, respectively. The total heterotrophic and bioluminescent *V. harveyi* counts were observed for every sampling. *V. harveyi* counts were observed in the VSHA medium under the darkroom. The maximum decrease on bioluminescent *V. harveyi* counts was observed on 5th, 10th, 15th, 20th, 25th, and 30th days and mean values for treatment were 4.60×10^4 CFU mL^{-1}, 1.81×10^4 CFU mL^{-1}, 4.90×10^3 CFUmL^{-1}, 4.70×10^3 CFU mL^{-1}, 6.80×10^3 CFU mL^{-1}, and 4.20×10^3 CFU mL^{-1}, respectively, as compare to control (1.17×10^5 CFU mL^{-1}, 2.62×10^5 CFU mL^{-1}, 7.40×10^4 CFU mL^{-1}, 2.02×10^4 CFU mL^{-1}, 2.53×10^4 CFU mL^{-1}, and 1.53×10^4 CFU mL^{-1}). Water quality parameters observed for every sampling are presented in Table 4. There were not many changes in water

Table 2 Molecular docking of biomolecules obtained from *S. costatum* extract against hemolysin protein of *V. harveyi* in comparison with control molecule Oxytetracycline dihydrate

Compounds	Binding energy (kcal/mol)	Intermolecular efficiency (kcal/mol)	Inhibition constant (mM)	H-bond interaction	Inhibitor atom	Amino acid residue	Distance of D & A (Å)	Hydrophobic interaction
Oxytetracycline dihydrate—control molecule	− 5.66	− 0.17	0.07	N–H….O O–H….O	O O	GLN210 NE2 ASP 390 OD2	3.01 3.09	LEU176; SER164; GLN165; ALA163; ASN162; TRP166; ASP390; TRP389; VAL391; ARG167; GLN210; GLY204; SER153; HIS393
1,2, benzene dicarboxylic acid	–	–	–	–	–	–	–	No interaction
3-Octadecanoic acid, methyl ester	− 3.15	− 0.16	4.94	N–H….O	O	ASN248 ND2	2.86	ALA163; SER153; SER164; GLN165; GLN210; TRP166; ARG167; HIS393; GLY204; ASN248
9-Octadecanoic acid, methyl ester	− 3.45	− 0.16	2.94	N–H….O N–H….O	O O	GLY204 N ASN248 ND2	2.79 2.94	ALA163; TRP166; ILE160; GLN210; ALA213; SER153; HIS393; GLY204; ASN248; LEU247
16-Octadecanoic acid, methyl ester	− 3.15	− 0.16	4.94	N–H….O	O	ASN248 ND2	2.86	GLN165; SER164; ALA163; TRP166; ARG167; HIS393, SER153; GLN210; GLY204; ASN248
Docasane	− 4.28	− 0.19	0.73	–	–	–	–	ALA213; ILE160; GLN210; SER153; HIS393; TRP166; ALA163; ASN162; SER164; GLN165

Table 2 (continued)

Compounds	Binding energy (kcal/mol)	Intermolecular efficiency (kcal/mol)	Inhibition constant (mM)	H-bond interaction	Inhibitor atom	Amino acid residue	Distance of D & A (Å)	Hydrophobic interaction
Heptadecanoic acid, methyl ester	− 3.69	− 0.18	1.99	–	–	–	–	ALA163; ASN162; TRP166; TRP389; ARG167; GLN210; ILE160; SER153; HIS393
n-hexadacanoic acid methyl ester	− 2.95	− 0.16	6.92	–	–	–	–	ALA163; TRP166; PHE161; ILE160; GLN210; ARG167; SER153; HIS393; TRP389
Octadecanoic acid	− 3.28	− 0.16	3.96	N–H….O N–H….O N–H….O O–H….O	O O O O	SER153 N GLY204 N HIS393 NE2 ASN248 OD1	3.19 3.10 2.99 2.78	ALA163; TRP166; GLN210; ARG167; HIS393; SER153; ASP152; GLY204; LEU247; ASN248
Pentadecanoic acid, 13 methyl, methyl ester	− 3.58	− 0.19	2.36	N–H….O N–H….O	O O	SER164 N GLN165 N	3.06 3.54	ALA163; GLN165; ASN162; SER164; ILE160; GLN210; SER153; ARG167; HIS393; TRP389
Tetradecanoic acid, methyl ester	− 3.67	− 0.22	2.02	N–H….O	O	SER 164: N	3.20	SER153; SER164; ALA163; GLN165; ASN162; TRP166; TRP389; ASP390; ARG167; HIS393

O oxygen, *N* nitrogen, *H* hydrogen, *D* donor, *A* acceptor of H ion, *SER* serine, *ALA* alanine, *ARG* arginine, *ASN* asparagine, *ASP* aspartic acid, *GLN* glutamine, *GLY* glycine, *HIS* histidine, *ILE* isoleucine, *LEU* leucine, *PHE* phenylalanine, *TRP* tryptophan, *VAL* valine

quality parameters both in treatment and control. The slight greenish color was observed as compared with control. However, other water quality parameters largely remained unaffected in treatment and control.

Effects of *S. costatum* Extract Against Growth and Virulence Factors of *V. harveyi* During Larviculture

During treatment, decreased growth of *V. harveyi* (OD) was observed for all the sampling days. During larviculture, *S.*

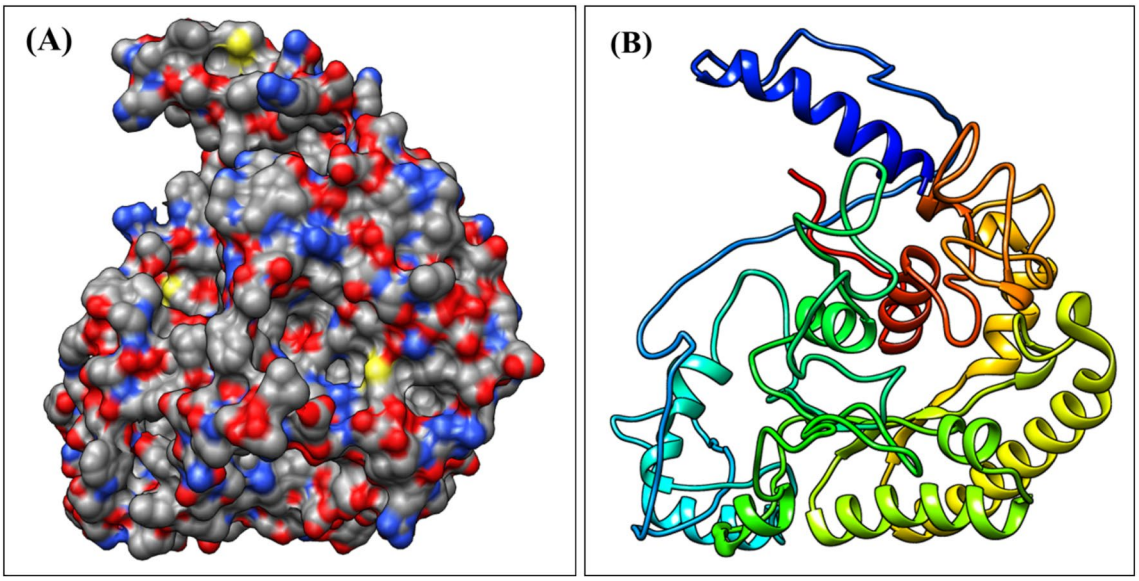

Fig. 1 **A** is representing the surface model (3-dimensional—3D) of *V. harveyi* hemolysin protein with different atom colors. **B** is denoting tertiary/skeletal structure or model of *V. harveyi* hemolysin protein. Here mentioned the different colors represent the name of the atom as follows: white mean Hydrogen, red means Oxygen, green means Fluorine/Chlorine, blue means Nitrogen, gray means Carbon, yellow means Sulfur, orange means Phosphorus, and other means varies—mostly dark red/pink/maroon (Color figure online)

costatum extract treatment controlled virulence factors such as luminescence, proteases, crude bacteriocin, cell surface hydrophobicity (SAT assay), hemolysis, and phospholipids of *V. harveyi* in all days of samplings. The results of growth and virulence factors affected by *S. costatum* extract during treatment against *V. harveyi* had shown in Table 5. The reductions of growth and virulence factors of *V. harveyi* were observed against *S. costatum* extract in all the sampling days during larviculture than control.

Statistical Analysis

The data assessment was made among the treatment and control. Significant differences were found ($P < 0.05$) between the *S. costatum* extracts treated with *V. harveyi* and control.

Discussion

The dry lab approach predicts the ligand orientation in a complex formed by the ligand itself with proteins or enzymes [15]. The quantification of the interaction is based on the shape and electrostatic interaction of the docked complex. Many docking programs (more than 50) and tools are now in use in the field of drug research [15]. Among them, AutoDock is one of the key tools being used in the field of drug development industries, etc. [15]. Therefore, the AutoDock tool was selected in the current study against *V. harveyi* hemolysin protein. Fatty acids play a main role in biological functions, such as membrane homeostasis, and metabolism, as signaling molecules. FadL is the only known protein that uptakes long-chain fatty acids in Gram-negative bacteria. Using *E. coli's* FadL as a template, *Vibrio cholera* FadL homologs vc1042, vc1043, and vca0862 have been folded, simulated on an atomistic level using molecular dynamics, and analyzed revealing the FadL transport channels. For vc1042 and vc1043 these transport channels have more structural accommodations for the many rigid unsaturated bonds of long-chain polyunsaturated fatty acids, while vca0862 was found to lack transport channels within the signature beta-barrel of FadL proteins [30]. Due to lack of an experimentally established crystal structure on the hemolysin protein produced by *V. harveyi,* the homology modeling may be best alternative method to construct a reasonable (3D) model of the target [31] which was followed here. The hemolysin is a pore-forming toxic protein produced by *V. harveyi*, which may cause lysis of RBC and other cells by producing spores on the cytoplasmic membrane. Therefore, it is considered the main virulence factor [32] and analyzed for molecular docking against marine algal inhibitors.

In the docking analysis, the ligand molecule (compounds/inhibitors) from *S. costatum* abiding passed Lipinski's rule of five, which showed its drug-likeness and the possibility of its considerations for further preclinical studies. Lead optimization of the marine algal compounds was known by the computation of drug-likeness properties. It was agreed with the report of Madeswaran et al. [33]. A lower docking

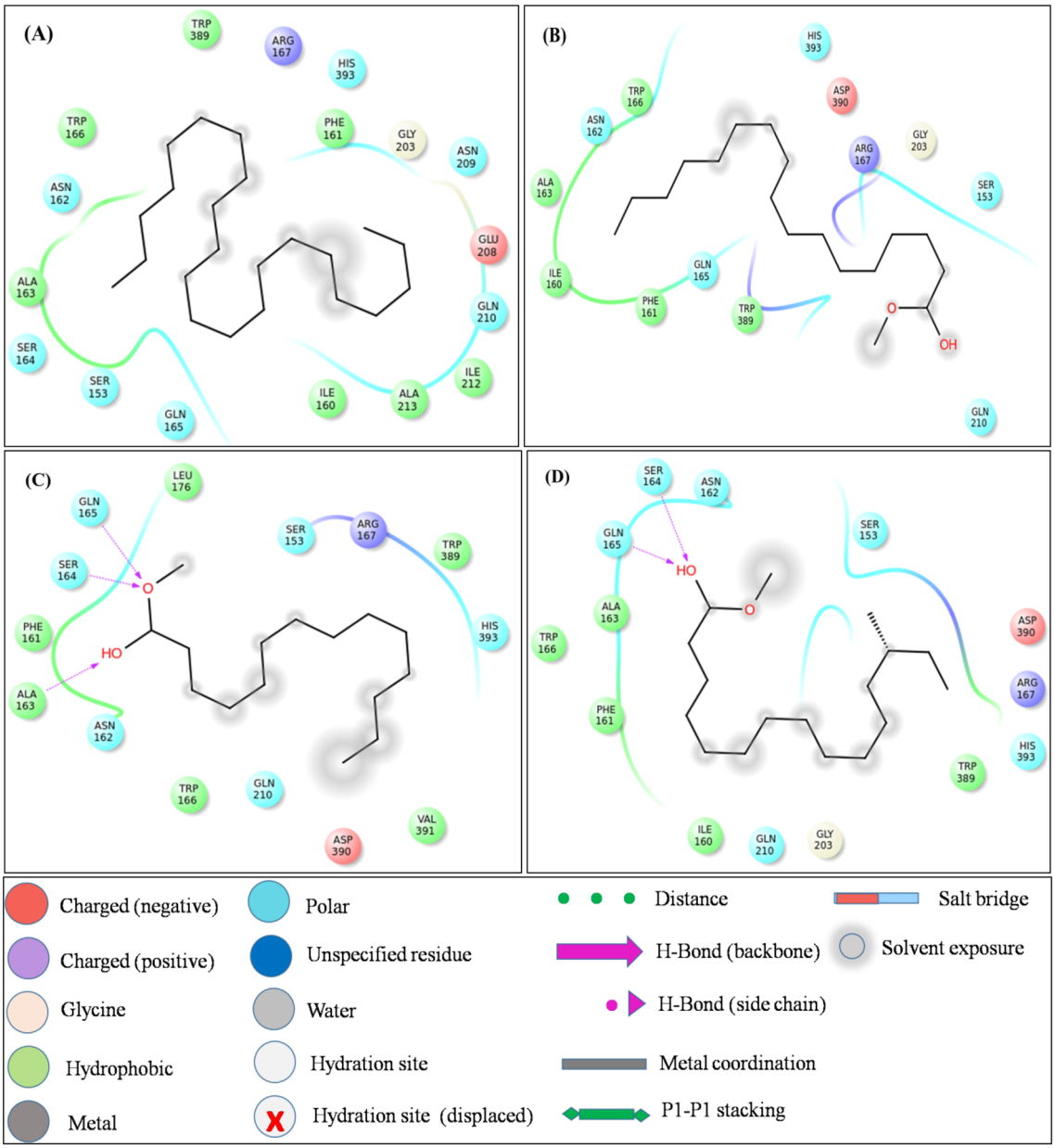

Fig. 2 The 2-dimensional (2D) view/pose of *V. harveyi* hemolysin protein was interacted/docked with molecules obtained by GC–MS analysis from the extract of *S. costatum,* in which only 9 molecules have docked with active site residues of hemolysin protein by hydrophobic or hydrogen bond or both interaction out of 10 compounds analyzed as follows: **A** Docosane, **B** Heptadecanoic acid, methyl ester, **C** Tetradecanoic acid, methyl ester, and **D** Pentadecanoic acid, 13 methyl, methyl ester

score exhibited higher binding affinity and is known as inhibiting biological activity [29]. The compounds from *S. costatum* extract were compared with the control molecule OTC by docking scores, such as binding energy, intermolecular efficiency, and inhibition constant. The *S. costatum* extract showed slightly higher docking scores as compared to OTC and also exhibited 90% of compounds docked with hemolysin protein. During, *P. monodon* larviculture, the *S. costatum* extract showed its non-existent level of inhibition or virulence of hemolysin produced by *V. harveyi* on sheep blood agar and improved survival of postlarvae than OTC and control. As a general rule, it was considered that in most of the potent inhibition mechanisms of compounds, both H-bond and hydrophobic interactions between the compound(s) and the active sites of the receptor are responsible for mediating biological activity [34]. Here, the compounds of *S. costatum* extract showed interactions through both H-bond and hydrophobic or either H-bond or hydrophobic on the active site residues (SER153, HIS393, ASP390) of hemolysin protein. The anticancer activity of

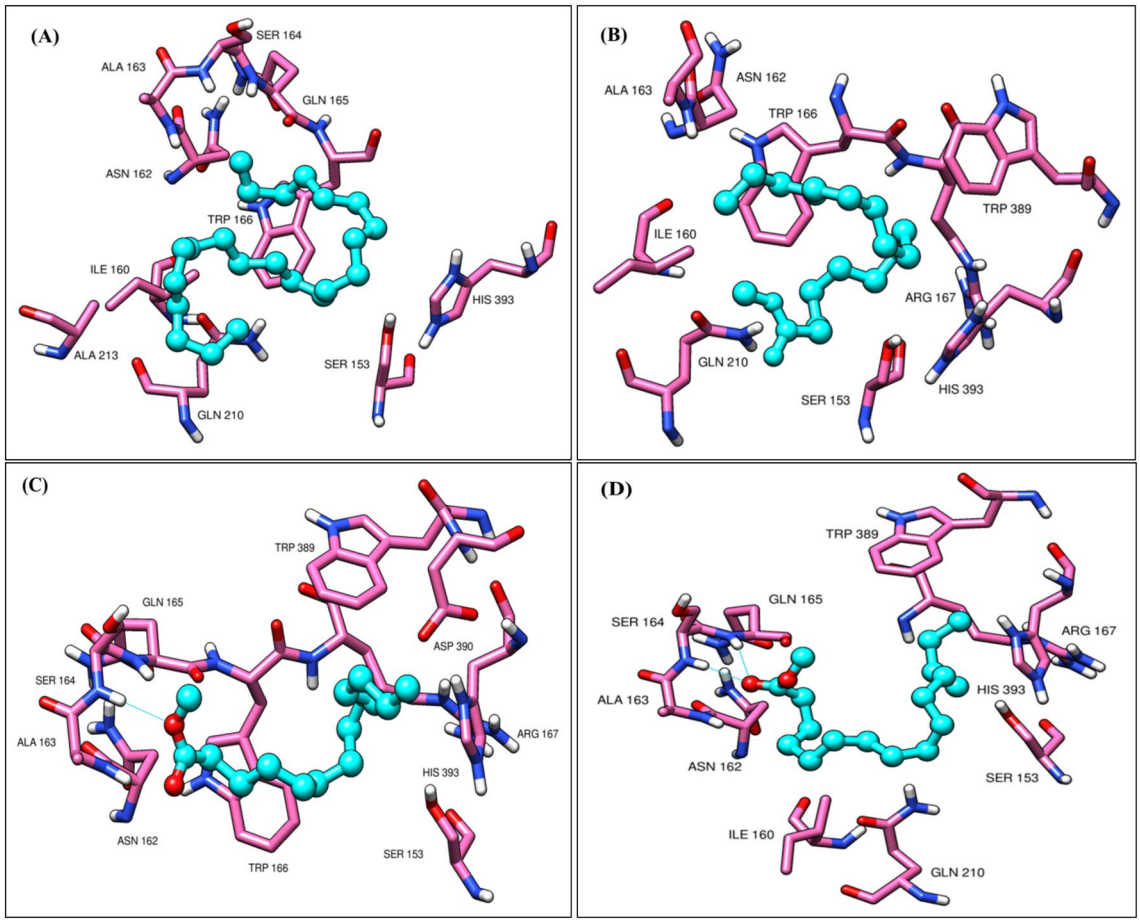

Fig. 3 The 3-dimensional (3D) view/pose of *V. harveyi* hemolysin protein was interacted/docked with molecules obtained by GC–MS analysis from the extract of *S. costatum*, in which only 9 molecules have docked with active site residues of hemolysin protein through hydrophobic or hydrogen bond or both interaction out of 10 compounds analyzed as follows: **A** Docosane, **B** Heptadecanoic acid, methyl ester, **C** Tetradecanoic acid, methyl ester, and **D** Pentadecanoic acid, 13 methyl, methyl ester

Table 3 Predicting drug-likeness of biomolecules from *S. costatum* based on the web tool by Supercomputing Facility for Bioinformatics and Computational Biology, IIT, New Delhi, India for Lipinski rule of five

Compounds	Molecular mass < 500 Dalton	High lipophilicity (expressed as LogP < 5)	Less than 5 hydrogen bond donors	Less than 10 hydrogen bond acceptors	Molar refractivity between 40 and 130
3-Octadecanoic acid, methyl ester	296.00	6.19	0	2	91.46
9-Octadecanoic acid, methyl ester	296.00	6.19	0	2	91.46
16-Octadecanoic acid, methyl ester	296.00	6.19	0	2	91.46
Docosane	310.00	8.82	0	0	103.68
Heptadecanoic acid, methyl ester	284.00	6.03	0	2	86.94
n-hexadacanoic acid methyl ester	270.00	5.64	0	2	82.32
Octadecanoic acid	284.00	6.33	1	2	87.18
Pentadecanoic acid, 13 methyl, methyl ester	270.00	5.49	0	2	82.25
Tetradecanoic acid, methyl ester	242.00	4.86	0	2	73.09

marine alga *Kappaphycus alvarezii* against cancer target by docking and found interaction with active site amino acid residues through hydrogen bond formation of *Homo sapiens* was studied by Shanker et al. [35]. It was concluded that

Table 4 Testing of *S. costatum* extract against *V. harveyi* with a reduction in cumulative percentage mortality during *P. monodon* larviculture

Parameters	Days						
	0	5th	10th	15th	20th	25th	30th
Cumulative percentage of mortality (CPM)							
Control (PL with *V. harveyi*)	0	13.66 ± 0.30	26.05 ± 0.90	35.63 ± 1.10	47.33 ± 1.50	62.13 ± 2.30	76.30 ± 2.90
Treatment (PL with extract and *V. harveyi*)	0	7.77 ± 0.20^{b}	16.56 ± 0.30^{b}	20.36 ± 0.50^{b}	27.05 ± 0.70^{b}	34.13 ± 1.10^{b}	41.10 ± 1.50^{b}
Control (PL with extract alone)	0	2.39 ± 0.10	6.19 ± 0.20	12.05 ± 0.50	18.13 ± 0.60	24.69 ± 0.90	29.56 ± 1.01
Control (PL without extract alone)	0	3.23 ± 0.10	6.03 ± 0.20	13.30 ± 0.50	17.43 ± 0.50	23.86 ± 1.01	28.39 ± 1.01
Treatment tubs (CFU mL^{-1})							
Total plate count	2.14×10^{6}	1.03×10^{5}	2.12×10^{5}	8.85×10^{4}	1.05×10^{4}	9.20×10^{4}	6.40×10^{4}
V. harveyi count	1.78×10^{6c}	4.60×10^{4b}	1.81×10^{4b}	4.90×10^{3b}	4.70×10^{3b}	6.80×10^{3b}	4.20×10^{3b}
Control tubs (CFU mL^{-1})							
Total plate count	2.59×10^{6}	1.45×10^{5}	2.74×10^{5}	1.46×10^{4}	7.80×10^{4}	1.88×10^{4}	2.06×10^{4}
V. harveyi count	1.86×10^{6}	1.17×10^{5}	2.62×10^{5}	7.40×10^{4}	2.02×10^{4}	2.53×10^{4}	1.53×10^{4}
The average weight of PL (mg)							
Treatment tubs	18.90 ± 3.01	67.50 ± 5.03	129.40 ± 4.01	168.50 ± 9.01	218.60 ± 11.01	249.70 ± 15.03	277.40 ± 10.03
Control tubs	18.30 ± 4.01	68.10 ± 5.03	133.60 ± 6.01	167.60 ± 8.01	217.70 ± 13.03	251.50 ± 13.01	279.80 ± 12.01
Water quality parameters (Treatment and Control)							
Temperature (°C)	29.00 ± 1.01	29.50 ± 1.01	29.00 ± 1.01	30.00 ± 0.81	30.00 ± 1.01	31.00 ± .0.91	30.00 ± 0.61
Salinity (PSU)	20.00 ± 0.51	20.00 ± 0.61	20.00 ± 0.41	21.00 ± 0.41	21.0 ± 0.61	21.00 ± 0.41	21.00 ± 0.71
pH in control tubs	8.40 ± 0.21	8.50 ± 0.11	8.40 ± 0.21	8.20 ± 0.33	8.00 ± 0.41	8.20 ± 0.41	8.10 ± 0.41
pH in treatment tubs	8.30 ± 0.21	8.40 ± 0.21	8.30 ± 0.21	8.50 ± 0.31	8.30 ± 0.21	8.10 ± 0.61	8.00 ± 0.21

Values are the average of three determinations with standard deviation (SD). PL: postlarvae, PSU: practical salinity units (1 PSU = 1 g/kg), Statistical analysis: a—($P < 0.01$); b – ($P < 0.05$); c—($P > 0.05$) or no significant differences. Among the cumulative percentage of mortality (CPM) values the significant difference was found at $P < 0.05$ level between in the control and treatment. Similarly the significant difference ($P < 0.05$) found between *V. harveyi* count between in control and treatment. But, the pH value and average weight of PL (mg) were found insignificantly between in control and treatments

analyzing the lowest binding energy (highly stable) of the ligand-binding complex exhibited using AutoDock tools will show the effective nature of inhibition of these receptors by the unique ligands. The biomolecule Methyl dehydroabietate showed the highest binding affinity among the compounds of *U. faciata* used in docking analysis against the hemolysin protein of *V. harveyi* was evaluated in a molecular docking study [25].

When the *S. costatum* extracts were tested, CPM reductions (35.20%) were noticed in *P. monodon* postlarvae, which were also supported by Navinar et al. [36] and who extracted lipophilic extracts from *S. costatum* which was inhibitory against the *Vibrio anguillarum* and *V. mytili* [36]. The *S. costatum* extract inhibited the growth of *V. alginolyticus* as a result of competitive exclusion [37]. The bacteria isolated from the marine diatoms have polyunsaturated aldehydes that antagonized 29 bacterial strains when it was tested [38]. Microalgae are potential sources for a wide range of PUFA, carotenoids, phycobiliproteins, polysaccharides, and phycotoxins [25] because lipids that inhibit microbes by disrupting cellular membranes [39] of bacteria, fungi, and yeasts. These fatty acids could further affect the expression of bacterial virulence factors, which are important for the establishment of infection. Therefore, in the present study, the level of *V. harveyi* was reduced in growth and also its virulence factors by treating against the crude extract of *S. costatum*. Further it was [39] proved that saturated and unsaturated fatty acids of algae can prevent initial bacterial adhesion and subsequent biofilm formation. Further, they postulated that fatty acids with chain lengths of more than 10 carbon atoms would induce the lysis of bacterial protoplasts [39]. *S. costatum* has indeed a high concentration of active lipids and fatty acids. This finding was corroborated by Selvin et al. [40]. Sivakumar et al. [25] was tested about the marine macro-alga *U. fasciata* extracts and found inhibitory against *V. harveyi* and its reductions against infection caused on *P. monodon* postlarvae and juveniles during larviculture. A computational methodology based on molecular docking and molecular dynamic simulations was used to find new microalgae metabolites inhibitors of β-lactamase. These results indicate that 4-Ph, Qn, and Crypt molecules, homologous from microalgae metabolites, could be used, likely as novel β-lactamase inhibitors or as for new in silico pharmaceutical designs, with the possibility of

Table 5 Testing of *S. costatum* extract against the growth and virulences produced by *V. harveyi* during *P. monodon* larviculture

Growth and virulence factors	Days					
	5th	10th	15th	20th	25th	30th
Luciferase (CPS)						
Treatment	103 ± 1.59[b]	114 ± 2.13[b]	117 ± 5.31[b]	111 ± 3.33[b]	111 ± 2.36[b]	100 ± 3.13[b]
Control	137 ± 4.16	123 ± 4.56	130 ± 5.63	148 ± 6.33	135 ± 3.69	129 ± 2.93
Salt aggregation test (SAT) (moles/L)[a]						
Treatment	1.50 ± 0.04[b]	2.00 ± 0.05[b]	1.75 ± 0.06[b]	2.00 ± 0.04[b]	2.50 ± 0.09[b]	2.00 ± 0.04[b]
Control	0.75 ± 0.03	0.50 ± 0.02	0.50 ± 0.01	0.75 ± 0.03	0.50 ± 0.02	0.50 ± 0.01
Bacteriocin OD at 660 nm						
Treatment	2.00 ± 0.05[b]	1.90 ± 0.01[b]	1.59 ± 0.05[b]	1.72 ± 0.04[b]	1.43 ± 0.03[b]	2.12 ± 0.09[b]
Control	2.12 ± 0.09	1.99 ± 0.06	2.16 ± 0.05	1.95 ± 0.01	1.96 ± 0.07	2.37 ± 0.11
Protease OD at 440 nm						
Treatment	0.14 ± 0.00[b]	0.11 ± 0.00[b]	0.12 ± 0.03[b]	0.12 ± 0.05[b]	0.16 ± 0.04[b]	0.15 ± 0.05[b]
Control	0.19 ± 0.05	0.17 ± 0.00	0.17 ± 0.06	0.14 ± 0.03	0.28 ± 0.01	0.36 ± 0.15
Growth OD at 600 nm						
Treatment	0.72 ± 0.02[b]	1.27 ± 0.02[b]	1.22 ± 0.03[b]	1.26 ± 0.01[b]	0.52 ± 0.02[b]	1.03 ± 0.02[b]
Control	1.03 ± 0.03	1.48 ± 0.05	1.65 ± 0.05	1.36 ± 0.04	0.77 ± 0.01	1.15 ± 0.05
Luminescence produced (CPS)						
Treatment	115 ± 2.36[b]	102 ± 1.63[b]	101 ± 2.16[b]	110 ± 4.03[b]	131 ± 3.79[b]	093 ± 3.01[b]
Control	136 ± 3.31	110 ± 4.01	118 ± 3.56	150 ± 5.06	154 ± 1.91	128 ± 2.63
Hemolytic activity[b]						
Treatment	–	–	–	–	–	–
Control	++++	++++	++++	++++	++++	++++
Phospholipase activity						
Treatment	–	–	–	–	–	–
Control	++++	++++	++++	++++	++++	++++

[a]SAT test (0.0 to 1.0 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)

[b]Qualitative activity of *V. harveyi;* − = non-existent, + = weak, ++ = moderate, +++ = high, ++++ = very high; Statistical analysis: a—($P < 0.01$); b—($P < 0.05$); and c—($P > 0.05$) or no significant differences. Among the tests of luciferase, Salt aggregation test, Bacteriocin OD, Protease OD, Growth OD, and Luminescence produced values, the significant differences were found at $P < 0.05$ level between the control and treatments

combating β-lactam resistance [41]. Besides, the hemolysin of *V. harveyi* is an important virulence determinant causing pathogenesis in marine fish, Turbot (*Scophthalmus maximus*), which was further characterized, and the enzyme was identified as a phospholipase B (Hemolysis activity). The site-directed mutagenesis revealed that a specific residue, SER153, was critical for its enzymatic activity and its virulence in fish [42]. In the present findings also we have found three active site residues in our structural template/model such as HIS393 and ASP390 including SER153 in the *V. harveyi* hemolysin protein.

Diterpene extracted from marine brown alga *Canistrocarpus cervicornis* caused anti-hemolytic activity as well as fibrinogen or plasma clotting induced by *Lachesis muta* snake venom protein [43]. The inhibition of snake venom protein from brown alga extract was compared with the antagonism of algal extracts against hemolysin protein. Not much data are available on fish or any aquatic resources to support this study. Further, anti-hemolytic activity exhibited by coriander (*Coriandrum sativum* L.) and filamentous green alga *Enteromorpha intestinalis* were reported against H_2O_2-induced hemolytic activity [44]. Hence, it was achieved due to the anti-hemolytic activity of filamentous green alga that was caused due to the compounds quenched H_2O_2 before it attacked the biomolecules of the erythrocyte membrane to causing oxidative hemolysis as reported by Costa et al. [45] on the effects of green tea.

Phycocyanin is the main pigment found in Micro-alga, *Spirulina platensis* and has the potential effect to treat effectively type 2 diabetes mellitus by inhibiting α-amylase and α-glucosidase. Molecular docking simulations indicated that phycocyanin inhibits the enzymes by binding to the active site and causing disruption in substrate-enzyme binding. Moreover, an in vitro inhibition activity test showed that

phycocyanin inhibits human salivary amylase by an average of 51.13% and it may contribute to making full use of phycocyanin as an anti-diabetic drug or therapeutic agent [46]. Hence, in the present study also Docasane and other eight inhibitors were bound with the active site of *V. harveyi* hemolysin protein and it could be the reason for the reduction of virulence factors in the in vitro and mortality of *P. monodon* postlarvae. Moreover, in the present findings, algal inhibitors of *S. costatum* occupied the active site of residues of hemolysin protein, before it acts upon the RBC members of phospholipid layers. Therefore, the compounds of *S. costatum* were performed as competitive to receptors for hemolysin protein and inhibited the hemolytic activity. The anti-hemolytic activity of flavonoid extract from the fruit peel of *Punica granatum* [47], spike, and fruit extracts of *Bombax ceiba pentandra* [48] was also studied.

Evaluation of seaweed sulfated polysaccharides as natural antagonists targeting *Salmonella typhi* OmpF was conducted using molecular docking and pharmacokinetic profiling. During this study, eleven compounds were targeted against *S. typhi* OmpF by the molecular docking approach and were compared with two commercially available typhoid medications. The sulfated polysaccharides showed good binding affinity compared to commercial drugs, particularly carrageenan/MIV-150, carrageenan lambda, fucoidan, and 3-phenyllactate, ranked as top antagonists against OmpF. Further, pharmacokinetics and toxicology studies corroborated that sulfated polysaccharides possessed drug-likeness and highly progressed in all parameters [49]. In our study also 10 compounds were targeted against the hemolysin protein of *V. harveyi* in the molecular docking analysis and found 9 compounds binding affinity that also passed the drug-likeness characteristics by Lipinski's rule of five. Further, *vhh* is an important virulence determinant of *V. harveyi*. It is an extracellular protein with phospholipase activity and plays a key role in the process of pathogen infection of the host [50]. The key role of *vhh* in the virulence of *V. harveyi* makes it an ideal target for the design of new antiviral drugs. It was found that gastrodin is a natural flavonoid with an effective antagonist of *vhh*-mediated hemolysis. The compound can effectively inhibit the damage caused by *V. harveyi* in cell culture and animal infection models. Gastrodin can downregulate the transcription of the *vhh* gene, and molecular modeling and mutation analysis show that it directly blocks the amino acid residues in the active center of the protein, thereby inhibiting its cytotoxicity [50]. Our research results also indicate that inhibitors identified from *S. costatum* are an effective antitoxin agent for *vhh*, which can be further developed into a new drug for the treatment of *Vibrio* infection. These findings were supported to compare with the present molecular docking studies from the extracts of *S. costatum* against the hemolysin protein of *V. harveyi* in aquaculture practices.

Conclusion

Generally, the docking results are effective not only for the pose construction and selection but also for virtual screening. There was no violation of the rule by the ligands used in docking for determining drug pharmacological activity. Besides in this study anti-hemolytic activity was observed against *S. costatum* extract. The docking scores from *S. costatum* showed inhibitory and likely in the larviculture testing. *S. costatum* extract also enhanced survival level on *P. monodon* postlarvae. The results of this study may facilitate to develop the new drugs or bio-agents to achieve sustainable aquaculture practices against *V. harveyi* and other aquaculture pathogens.

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Author Contributions KS and SK designed research and wrote the manuscript. KS and BV performed docking work and analyzed data. KS and BV reviewed and edited the manuscript.

Declarations

Conflict of interest The authors have no conflicts of interest to declare that are relevant to the content of the article.

Ethical Statement This article does not contain any study with vertebrate animals, thus it was required no animal ethic statement.

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