Molecular docking study of bio-inhibitors extracted from marine macro-alga *Ulva fasciata* against hemolysin protein of luminescence disease-causing *Vibrio harveyi*

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**Abstract**

Shrimp grow-out and hatchery systems are being affected by bacterial disease particularly *Vibrios*. The use of chemotherapeutic agents in aquaculture practices has led to the development of resistance among aquatic bacteria. Thus, health management becomes of major importance in aquaculture. Under this situation, progressing bio-inhibitors from marine resources are most appropriate to be considered against pathogenic bacteria. Molecular docking is an appropriate tool in structural biology and computer-assisted drug design to predict and neutralize a target protein of known diseases. In this study, marine macro-alga *Ulva fasciata* was aimed at developing inhibitors against luminescence disease-causing pathogenic bacteria *Vibrio harveyi*. *U. fasciata* was collected from Thoothukudi, Tamil Nadu, India. Extract of *U. fasciata* was tested against growth and virulence factors of *V. harveyi* during *Penaeus monodon* larviculture. Further *U. fasciata* extract was subjected to GC–MS analysis to identify the biomolecules. The homology modeling of virulent protein, hemolysin of *V. harveyi* was designed in this study. Hence, it was aimed for molecular docking against the biomolecules identified from *U. fasciata* extract. During shrimp larviculture, the extract of *U. fasciata* (200 μg mL⁻¹) exhibited reduction on Cumulative Percentage of Mortality (32.40%) in postlarvae against challenge of *V. harveyi* infection. Biomolecule Methyl dehydroabietate had showed highest binding affinity among the compounds was evaluated in molecular docking study. Statistical analysis had revealed significant differences (p < 0.05) in trials. Therefore, it was proved that the bio-inhibitors from *U. fasciata* will be a better option for controlling luminescence disease-causing *V. harveyi* in shrimp grow-out practices.

**Keywords** *Ulva fasciata* · Bio-inhibitors · Molecular docking · *Vibrio harveyi* · Hemolysin protein · Shrimp larviculture

**Introduction**

The grow-out practices under the aquaculture sector are major sources in generating job opportunities and revenue to business sectors. The demand for seafood is being increased...
throughout the years. The shrimp grow-out practices have developed rapidly across the world due to the increased market demand (Harlioglu and Farhadi 2017). In shrimp grow-out system, disease problem that leads to severe economic losses worldwide. While considering bacterial diseases in shrimp culture Vibriobacteria producing disease is known as Vibriosis a major contributorto other disease-causing agents (Krupesha-Sharma et al. 2016). Among other Vibriobacteria, V. harveyi a bioluminescent bacterium, is a potent pathogen and resulting in major mortalities in grow-out and hatchery units. During its pathogenesis in host organisms, V. harveyi produces many extra-cellular virulence factors, such as bioluminescence, proteases, phospholipases, lipases, siderophores, chitinases, hemolysins, etc. (Soto-Rodriguez et al. 2012). Among other virulence factors, hemolysin is the most widely distributed exotoxin produced by the V. harveyi (Gavin et al. 2017). Further, hemolysins are capable to lyse erythrocytes and achieve two different modes of action, including cell pore-forming protein and phospholipase enzyme activity (Basso et al. 2017). Virulent isolates of V. harveyi were recognized for expressing vhh gene during infection of brine shrimp larvae whereas non-virulent isolates showed lesser expression of vhh gene (Ruwandeepika et al. 2012). V. harveyi virulent strain was found to contain two copies of the hemolysin gene (Zhang et al. 2001) which was infected salmon fish. Moreover, V. harveyi is associated with other luminescent Vibrios (Raissy et al. 2011) to spread diseases during the grow-out practices of shrimp Penaeus monodon and Litopenaeus vannamei. Most findings also suggested that larval and postlarval stages are more susceptible to bacterial infection than the juvenile and adult stages of shrimp (Runggrassamee et al. 2013). However, the mass mortalities resulting infections by V. harveyi were reported up to 80–100% in shrimp grow-out and larviculture system (Raissy et al. 2011).

Monitoring bacterial diseases in aquaculture practices, through the application of antimicrobial chemicals, has led to the emergence of more resistance among the bacterial pathogens (Chen et al. 2018). Under these circumstances, it is of vital importance to develop alternative agents which is sustainable and eco-friendly. While considering the modern drug designing industries, molecular docking has become a key tool in structural biology and computer-assisted drug design against the disease of our interest (de Ruyck et al. 2016). Generally, docking is done between a small molecule and a target macromolecule. It is often referred to as ligand–protein docking (Morris and Lim-Wilby 2008) aimed at predicting the predominant binding mode(s) of a ligand with a protein of known three-dimensional (3D) structure. Due to the lack of an experimentally established crystal structure of a given protein, homology modeling is the alternative to construct a reasonable (3D) model of the target (Bordoli et al. 2009). This model will be appropriate for applications like agonists or antagonists (Li and Wang 2007). Moreover, by generating binding energies in these docking studies, the position of the ligand in the enzyme-binding site can be visualized (Wisastra et al. 2013).

Marine resources are an unmatchable reservoir of biologically active natural products, many of which exhibit structural features on par with the terrestrial organism (Saritha et al. 2013). Polysaccharides such as alginate, carrageenan produced by marine macroalgae were capable of improving the health status of marine fish (Peso-Echarri et al. 2012). Ulva fasciata is a more commonly available marine green macroalga (Chlorophyceae), which grows abundantly in both intertidal zone of the sea, and is recognized as an important source for antioxidant (Chakraborty and Paulraj 2010). Also, the extracts from U. reticulate and U. lactuca were proved for antagonism against many human pathogens (Kolanjinathan and Stella 2011). The extract of U. fasciata shows inhibition against aquatic bacterial pathogens in laboratory trials (Priyadharshini et al. 2011). The efficacy of U. fasciata incorporated diet was also proved in controlling shrimp bacterial pathogens, such as V. fischeri, V. alginolyticus, and V. harveyi, during the infection on P. monodon postlarvae (Selvin et al. 2011). Several herbal plant diets were fed to P. monodon postlarvae and tested against V. harveyi, the results showed 75% improvement in survival (Velmurugan et al. 2010). Also, the reduced mortalities on P. monodon postlarvae tested against V. harveyi was observed during the oral administration of fucoidan compound isolated from brown algae Undaria pinnatifida through feed (Traifalgar et al. 2009). The extract from brown alga Endarachne binghamiae which was administered to L. vannamei postlarvae (PL10) through Artemia nauplii and observed enhanced survival against V. alginolyticus infection (Wong et al. 2008). When V. harveyi was tested against P. monodon postlarvae (PL15), and fed with Artemia diet enriched by herbal products had found improved survival on postlarvae (Immanuel et al. 2007). Under this situation, it is crucial to know the pathogenic-cum-virulence mechanisms of V. harveyi and constitute different bio-inhibitors which would enable the beneficial approach through molecular docking analysis for controlling of V. harveyi in shrimp grow-out systems.

Materials and methods

Isolation of V. harveyi strain

The V. harveyi strains were isolated from the infected P. monodon larviculture tanks. It was identified using standard morphological and biochemical tests (Abraham and Palaniappan 2004). The strains were pre-enriched in alkaline peptone water (APW) and serially diluted with normal
saline (0.85% NaCl w/v), 0.1 ml of each sample was surface spread on Thiosulphate citrate bile salt sucrose agar medium (TCBS), Sea water complex agar (SWC) and V. harveyi selective agar medium (VHSA). In SWC agar, bio-luminous colonies were incubated at 30 °C/20 h and observed under darkroom. The isolates were compared with V. harveyi ATCC 25919 strain and then re-confirmed by PCR test (Maiti et al. 2009). The pathogenicity of V. harveyi was ascertained by spotting in 3% blood agar (Rattanama et al. 2012).

Collection of marine macroalga, extraction of compounds and estimation of MIC

The marine green macroalga U. fasciata was collected from the intertidal zone of Thoothukudi coastal area (Latitude 8.7874°N; Longitude 78.1983°E) of Tamil Nadu, India. It was washed in freshwater and 10 mg L⁻¹ (w/v) of KMnO₄ solution to remove epiphytes, sand, and other extraneous matters, later dried under shady condition. The algae were then weighed, pulverized by mechanical grinder and subjected to extraction using ethyl acetate solvent for obtaining compounds by cold extraction method. During extraction, 1 g of alga powder was mixed with 10 ml of solvent. It was incubated at 30 °C/96 h at 50 rpm. Later, the extract was filtered by Whatman filter paper grade No.1. The filtered extract was evaporated at 30 °C under vacuum and further stored at 4 °C for further use (Das et al. 2005). The resultant extract was liquefied at 5 mg per mL of 30% (v/v) DMSO (Dimethyl Sulfoxide). The estimation of minimum inhibitory concentration (MIC) of U. fasciata extract against to the V. harveyi was evaluated as described by Islam et al. (2008).

Preparation of inhibitors data

The biomolecules/bio-inhibitors/ligand molecules/compounds were identified by Gas Chromatography and Mass Spectrometry (GC–MS) analysis (Sivakumar et al. 2014) from the extract of U. fasciata. It was used as ligand molecules in the present docking studies. PUBCHEM (http://pubchem.ncbi.nlm.nih.gov)—PubChem is a 3D structural database, organized as three linked databases within the NCBI’s Entrez information retrieval system. The 3D structure of compounds was downloaded from this database based on GC–MS results obtained. These 3D structures were further converted to PDB (Protein Data Bank) format using PyMOL (0.99rc6) software (Saeed et al. 2011). These PDB files were used for the docking study.

Homology modeling of hemolysin protein

The 3D structure of the hemolysin protein of V. harveyi (as of June 2014) was not available in the Protein Data Bank (PDB; http://www.pdb.org). The primary sequence of hemolysin protein of V. harveyi was obtained from NCBI through Protein Sequence ID AAG25957.1. However, there was no suitable template/model available for hemolysin of V. harveyi. Therefore, the sequence was used to build the 3D model of protein structure using Phyre² (Protein Homology/AnalogY Recognition Engine, Version 2.0) (Rounak et al. 2014). 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 (Kelley and Sternberg 2009). The percentage of identity is 37.1% between the sequences in homology modeling. Phi/Psi dihedral angle for the predicted model was validated using Ramachandran plot from PROCHECK (Laskowski et al. 1993). The modeled structure has 5.29% of amino acid residues in outliers of the Ramachandran plot.

Preparation of hemolysin protein and active-site prediction

The structure modification was performed in the hemolysin protein for docking studies like the addition of hydrogen atoms, assigning correct bond orders, fixing of the charges and orientation of groups. Following this, optimization of the amino acid orientation of hydroxyl groups, 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 the default value of 0.3 Å (Parasuraman et al. 2012). In docking analysis, the active site/binding site of hemolysin protein was recognized 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 (Van den Berg 2010).

Docking protocol

In the current study, the 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). During docking, an extended PDBQT format of PDB file was used for coordinate files, which include atomic partial charges. All the hydrogen atoms of the macro-molecule (protein) were added using the AutoDockTools software (Version 1.5.2 revision 2) for preparation of the target protein hemolysin (unbound target), which is an essential step for the correct calculation of partial atomic charges. Kollman charges were calculated for each atom of the macromolecule in AutoDock 4.0. In docking, the grid dimensions were 60×60×60 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 computation of drug-likeness properties. The drug-likeness scores of the compounds were evaluated with the help of Lipinski’s rule. During the docking process, a maximum of 10 conformers or poses were considered for each compound. The best interaction(s) pose was showed by protein and ligand molecule along with least binding energy exhibited (kcal mol⁻¹) that was considered as the highest inhibiting activity of the respective compound and compared to the control molecule Oxytetracycline dihydrate (OTC) (Hi-Media, India). The binding energy of the individual compound was decided based on the amount of free energy required, for 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 (nM) and intermolecular efficiency (kcal mol⁻¹), were also measured along with binding energy exhibited, since they are directly proportional to binding energy (Madeswaran et al. 2012; Tomi et al. 2016). PyMOL and UCSF Chimera (Version 1.8.1) software (Pathak et al. 2014) were used for graphical visualization, analyzing hydrogen bond interactions, and producing quality images. Hydrophobic interactions were observed between protein and ligand using Chimera software. All the docking calculations were performed in Intel(R) Core™ i3-2310 M CPU at 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.

**Predicting drug-likeness of the compounds**

An online software SwissADME web tool (http://www.swissadme.ch/) was used to retrieve the information about drug-likeness properties of biomolecules with the help of Lipinski rule of five (Daina et al. 2017). Lipinski rule helps to distinguish drug and non-drug like properties of molecules. It is aided to identify the possibility of success or failure due to drug-likeness for molecules by fulfilling with three or more of the following rules; (i) Molecular mass < 500 Dalton (ii) High Lipophilicity (expressed as LogP < 5) (iii) Less than 5 hydrogen bond donors (iv) Less than 10 hydrogen bond acceptors (v) Molar refractivity between 40 and 130 (Daina et al. 2017).

**Larviculture trial of *U. fasciata* extract against *V. harveyi***

The experimental tubs were washed with 10 mg L⁻¹ KMnO₄ solution (w/v) and filled with 20 L of saline water at 20 ppt (Parts per thousand). *P. monodon* disease-free postlarvae (PL15) (Ananda Raja et al. 2017) (after checking OIE listed diseases) were procured from the commercial Shrimp Hatchery located at Chennai, India. These postlarvae were acclimatized at 20 ppt for 5 days at 29 ± 1 °C with sufficient aeration. In each tub, the average weight of postlarvae of 17 to 18 mg was stocked at 1000 numbers. The control tub was inoculated with *V. harveyi* (10 mL of 1.80 OD) and PL. In treatment tub, the PL was inoculated with *V. harveyi* and 200 μg of extract of *U. fasciata* per mL of saline water (2 g/10L). Another control as reference tub was added extract (200 μg mL⁻¹) alone and PL. The 4th tub was an additional reference control for PL alone without *U. fasciata* extract and *V. harveyi*. The fifth tub was known as a reference control for extract, it was added with *V. harveyi* and OTC (200 μg mL⁻¹). The sufficient level of dissolved oxygen was maintained by aeration in each tub. The feed was provided to PL daily two times (morning and evening) at 5% body weight. The water quality parameters were examined daily, such as temperature, salinity, and pH. The mortality of PL was noted every day. The water exchange was not given for all the tubs for 30-day experimental period, but the evaporated water was filled with sterile saline water. The sample water from the each experimental tub was collected once every 5 days using sterile water bottles. The total *V. harveyi* counts (Kannappan et al. 2013) and heterotrophic bacteria (Kannappan et al. 2013) were enumerated with VHSA and SWC medium correspondingly by the spread plate technique. The experimental tubs were provided with shelters on top to avoid the possibility of external contamination. Triplet was performed for each experiment. The mean values were represented in standard deviation. The value of cumulative percentage of mortality (CPM) in *P. monodon* larviculture was noted at end of 30-day trial (Kannappan et al. 2013).

**Effect of *U. fasciata* extract against growth and virulence factors of *V. harveyi* during *P. monodon* larviculture trial**

The effect of *U. fasciata* extract on virulence factors produced by *V. harveyi* was observed during the experiment for every 5 days. The luminous *V. harveyi* colonies were examined from *U. fasciata* extract treatments by VHSA medium, which was later inoculated into LB (Luria Bertani) broth (Hi-Media, India) and incubated at 28 °C/100 rpm/24 h. The spent culture of *V. harveyi* was taken. It was used to evaluate the growth and various virulence factors produced.
by *V. harveyi*, such as luciferase and luminescence production (Luciferase assay kit—LUC1, Technical Bulletin MB-260, Sigma, USA), hemolytic activity, phospholipase activity, extracellular crude protein (Bacteriocin), protease enzyme production and growth of *V. harveyi*, were measured as described by Soto-Rodriguez et al. (2012); Kannappan et al. (2013); MadhusudanaRao et al. (2013). Also, cell surface hydrophobicity was examined by Salt aggregation test (SAT) (Soto-Rodriguez et al. 2012; MadhusudanaRao et al. 2013). The results of the agar plate assay were evaluated and graded based on the hydrolysis of the medium around the inoculated colonies. Thus, the activity was coded by qualitative parameters like non-existent (−), weak (+), moderate (++), high (+++), and very high (++++) (Soto-Rodriguez et al. 2003). Each test was performed in triplicates and the mean value was expressed with standard deviation. One factor analysis of variance was used for statistical evaluation using SPSS ver. 16.0 software to assess the significance in the present study.

**Results**

**Identification, characterization and MIC of *V. harveyi***

All the *Vibrio* bacteria isolates were known as *V. harveyi*, since they were Gram-negative rods, motile, oxidase-positive, fermented glucose and were sensitive to the vibrio-static agent O/129 and used D-mannitol as a sole source of carbon and made bioluminescence as observed in the darkroom. The isolates of *V. harveyi* obtained (negative for Voges–Proskauer, citrate positive, positive growth with 8% NaCl and negative with 0% NaCl) were stored in LB broth containing sterile glycerol (15% v/v). Further, the PCR assay was performed for the expected fragment size of 235 bp of *vhh* gene indicating the presence of hemolysin among the isolates were tested. The *V. harveyi* bacteria isolates confirmed were named as (Vh1 to Vh20), *V. harveyi* strain Vh1 was used in the present study. In SWC agar, bio-luminous colonies of *V. harveyi* were observed at 30 °C/20 h under darkroom (Fig. 1). The hemolytic activity of *V. harveyi* by spotting in sheep blood agar was examined (Fig. 2). The MIC of *U. fasciata* extract was observed at 30 µg concentration against the *V. harveyi*.

**Effect of bio-inhibitors from *U. fasciata* extract against hemolysin protein of *V. harveyi* in molecular docking study**

The biomolecules were identified by GC–MS analysis from extract of *U. fasciata* was shown in Fig. 3 and Table 1. GC–MS analysis has revealed totally 36 compounds. The different structure/model of hemolysin protein of *V. harveyi* and its best interaction pose formed with OTC as depicted (Fig. 4). Based on the results observed from the molecular docking (Table 2), the best leading three compounds were selected (Fig. 5a–c) from the inhibitors of *U. fasciata*. Totally 36 compounds from *U. fasciata* were subjected to the docking analysis and found interactions only with 18 compounds by exhibiting H-bonding or hydrophobic interactions or both with three important active-site residues (SER153, HIS393, and...
ASP390) including other residues of hemolysin protein of V. harveyi. The remaining 18 compounds did not show such interaction with active-site residues of V. harveyi. Among the inhibitors docked from U. fasciata, the inhibitor Methyl dehydroabietate showed highest binding activity by exhibiting docking scores like lowest binding energy (-6.46 kcal mol\(^{-1}\)), inhibition constant (0.018 mM), and intermolecular efficiency (-0.28 kcal mol\(^{-1}\)) followed by inhibitor Bis(2-ethylhexyl) phthalate of binding energy (-5.98 kcal mol\(^{-1}\)), inhibition constant (0.041 mM) and intermolecular efficiency (-0.21 kcal mol\(^{-1}\)), and inhibitor 1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester of binding energy (-5.77 kcal mol\(^{-1}\)), inhibition constant (0.058 mM) and intermolecular efficiency (-0.24 kcal mol\(^{-1}\)), respectively, when compared to control molecule OTC of binding energy (-5.66 kcal mol\(^{-1}\)), inhibition constant (0.071 mM) and intermolecular efficiency (-0.17 kcal mol\(^{-1}\)). Moreover, the best leading three compounds, such as Methyl dehydroabietate, Bis(2-ethylhexyl) phthalate and 1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester, have showed binding affinity by both hydrogen bond and hydrophobic interactions. Phthalic acid, 2-ethylhexyl pentyl ester has showed only hydrophobic interactions with following better docking scores of binding energy (-5.68 kcal mol\(^{-1}\)), inhibition constant (0.069 mM) and intermolecular efficiency (-0.23 kcal mol\(^{-1}\)) than control molecule OTC. Hence, in the present study, only four compounds have exhibited better docking scores than the control molecule. However, 18 compounds of U. fasciata have showed binding affinity with hemolysin protein in the present docking. Further, 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 (SwissADME web tool) analysis exhibited that all the 18 compounds of U. fasciata possessed drug-likeness properties (Table 3).

**Evaluating of U. fasciata extract against V. harveyi in P. monodon larviculture**

In P. monodon, larviculture trial found decline of CPM at 32.40% in PL as compared to the control (76.30%) by U. fasciata extract treatment with V. harveyi. Other reference controls, the results on CPM in PL for extract were 29.56%, with PL alone was 28.39%, whereas, in OTC trial, it was 46.80%. In PL average body weight, there were not much of differences observed among treatment and control and it had been recorded as follows of 269.3 mg and 266.5 mg for control and treatment, respectively, in end of the trial. The maximum decline on V. harveyi counts was observed on the 5th, 10th, 15th and 20th days and the mean values for treatment were 2.38 × 10^4, 1.56 × 10^4, 4.30 × 10^3 and 3.40 × 10^3 CFU mL\(^{-1}\) as compared to control which is 3.40 × 10^5, 1.44 × 10^5, 1.45 × 10^5 and 2.49 × 10^4 CFU mL\(^{-1}\) correspondingly. The recorded
water quality parameters like temperature, salinity, and pH were in every sampling shown in Table 4. During larviculture, the temperature range was recorded at minimum of 29 °C and maximum of 31 °C. Similarly, the range of salinity was observed at 20–21 ppt. Water pH was measured minimum at 8.1 and maximum at 8.5 for control tanks, in the case of treatment tank, the minimum pH was noted at 8.0 and maximum at 8.5. Therefore, it was observed that there was not much of variation of water quality parameters among control and treatment. Although, the light greenish color was observed in the

| GC–MS profile of *U. fasciata* extract |
|--------------------------------------|
| Retention time (min) | Name of the compound | Peak area (%) | Molecular formula | Molecular weight |
|----------------------|----------------------|---------------|-------------------|-----------------|
| 4.03                 | Anisole              | 0.80          | C₇H₈O             | 108.13          |
| 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          | C₁₄H₁₉NS           | 135.18          |
| 11.67                | 1-Tetradecene        | 0.52          | C₁₄H₂₈             | 196.37          |
| 11.77                | Tetradecane          | 0.07          | C₁₄H₃₀             | 198.38          |
| 13.15                | Phenol, 2,4-bis(1,1-dimethyl) | 0.34 | C₁₄H₂₂O | 206.32          |
| 14.21                | Cetene               | 0.60          | C₁₆H₃₂             | 224.42          |
| 14.29                | Hentriacontane       | 0.10          | C₁₅H₂₆             | 436.83          |
| 15.21                | 8-Heptadecene        | 0.23          | C₁₇H₃₄             | 238.45          |
| 15.44                | Heptadecane          | 0.06          | C₁₇H₃₆             | 240.46          |
| 15.70                | Phenol, 2-(1-phenylethyl)- | 0.19 | C₁₄H₁₄O | 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-dimethyl- | 0.13 | C₁₃H₂₆O | 198.34          |
| 17.18                | Dibutyl phthalate    | 0.30          | C₁₆H₃₂O₂           | 278.34          |
| 17.36                | Phytol, acetate      | 0.11          | C₂₂H₄₅O₂           | 338.56          |
| 17.66                | Phthalic acid, butyl isohexyl ester | 0.45 | C₁₈H₃₆O₄ | 306.39          |
| 17.84                | Phthalic acid, 2-ethylhexyl pentyl ester | 0.09 | C₂₁H₃₂O₄ | 348.47          |
| 18.14                | 1,2-Benzenedicarboxylic acid, butyl | 2.47 | C₂₀H₃₀O₄ | 334.44          |
| 18.32                | Phthalic acid, butyl isohexyl ester | 1.12 | C₁₈H₃₄O₄ | 306.39          |
| 18.51                | 5-Eicosene, (E)-      | 0.66          | C₂₀H₄₀             | 280.53          |
| 18.56                | Dodecane, 1,1'-oxybis- | 0.10 | C₂₄H₃₄O₂ | 354.65          |
| 19.89                | Silanetriamine, 1-azido-N,N,N',N'',N''',N''''-hexamethyl- | 0.21 | C₆H₁₈N₆Si | 202.33          |
| 20.36                | Behenic alcohol      | 0.26          | C₂₂H₄₆O₂           | 326.60          |
| 20.87                | 1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester | 0.27 | C₂₀H₃₀O₄ | 334.44          |
| 21.70                | Methyl dehydroabietate | 0.10 | C₁₃H₂₆O₂ | 314.46          |
| 22.08                | Octacosanol          | 0.12          | C₂₈H₅₈O₂           | 410.75          |
| 22.24                | Phenol, 2,4-bis(1-phenylethyl)- | 0.25 | C₁₀H₂₈N₂O₃ | 479.56          |
| 22.37                | Phenol, 2,4-bis(1-phenylethyl)- | 0.49 | C₁₀H₂₈N₂O₃ | 479.56          |
| 22.80                | Bis(2-ethylhexyl) phthalate | 0.45 | C₂₄H₃₈O₄ | 390.55          |
| 23.02                | Naphthalene, 6-chloro-1-nitro- | 0.13 | C₁₀H₆CINO₂ | 207.61          |
| 23.21                | Bis(2-ethylhexyl) phthalate | 88.42 | C₂₄H₃₈O₄ | 390.55          |
| 26.89                | Benzo[h]quinoline, 2,4-dimethyl- | 0.22 | C₁₅H₁₄N | 207.27          |
Fig. 4 The tertiary structure (a) and its surface model (b) of hemolysin protein. The best-docked pose formed by OTC showed in (c) tertiary structure, (d) surface model with atoms color, (e) without atoms color, and (f) close view of docked with hemolysin protein.
Table 2  Molecular docking parameters of bio-inhibitors from *U. fasciata* against hemolysin protein of *V. harveyi* in comparison with control molecule Oxytetracycline dihydrate

| Compounds                                | Binding energy (kcal mol⁻¹) | Intermolecular efficiency (kcal mol⁻¹) | 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                                | 0.071                    | N–H….O            | O              | GLN210 NE2        | 3.013                | LEU176; SER164; GLN165; ALA163; ASN162; TRP166; ASP390; TRP389; VAL391; ARG167; GLN210; GLY204; SER153; HIS393 |
| 1,2-Benzenedicarboxylic acid, butyl       | -4.31                      | -0.18                                | 0.697                    | NH….O             | O              | GLN210 NE2        | 2.689                | TRP166; TRP389; ASP390; VAL391; HIS393; ARG167; SER153; GLN210; ILE160 |
| 1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester | -5.77                      | -0.24                                | 0.058                    | N–H….O            | O              | GLN210 NE2        | 2.971                | GLN210; ASP156; ALA163; TRP166; SER153; GLY204; ASN248; HIS393; ARG167 |
| 1-Decene                                  | -3.95                      | -0.22                                | 1.270                    |                    |                |                   |                      | LEU176; TRP166; TRP389; ARG167; ASP390; HIS393; SER153; ILE160; GLN210 |
| 1-Octadecene                              |                            |                                      |                          |                    |                |                   |                      | No interaction |
| 1-Tetradecene                             |                            |                                      |                          |                    |                |                   |                      | No interaction |
| 2-Undecanone, 6,10-dimethyl-              |                            |                                      |                          |                    |                |                   |                      | No interaction |
| 5-Eicosene, (E)-                          | -4.38                      | -0.22                                | 0.618                    |                    |                |                   |                      | ALA163; TRP166; TRP389; ILE160; GLN165; GLN210; SER164; SER153; HIS393; ARG167 |
| 5-Tetradecene, (E)-                      |                            |                                      |                          |                    |                |                   |                      | No interaction |
| 8-Heptadecene                             | -4.29                      | -0.25                                | 0.716                    |                    |                |                   |                      | ARG167; TRP166; SER153; GLN210; ILE160 |
| Anisole                                   |                            |                                      |                          |                    |                |                   |                      | No interaction |
| Behenic alcohol                           | -3.89                      | -0.17                                | 1.41                     | N–H….O            | O              | HIS393 NE2        | 2.783                | ALA163; TRP166; ILE160; GLN210; ASP152; GLY204; ASN248; LEU247; HIS393; LEU154; SER153 |
| Benzo[hi]quinoline, 2,4-dimethyl-         |                            |                                      |                          |                    |                |                   |                      | No interaction |
| Benzothiazole                              |                            |                                      |                          |                    |                |                   |                      | No interaction |
| Bicyclo[3.1.1]heptanes, 2,6,6-trimethyl-, (1.alpha.,2.beta.,5.alpha.) | | | | | | | | |
| Bis(2-ethylhexyl) phthalate               | -5.98                      | -0.21                                | 0.041                    | O–H….O            | O              | SER153 OG         | 3.232                | ALA163; SER164; GLN165; TRP166; ILE160; GLN210; TRP389; SER364; SER365; ASP390; VAL391; HIS393; ARG167; SER153 |
| Benzene                                   |                            |                                      |                          |                    |                |                   |                      | No interaction |
| Dibutyl phthalate                         | -4.43                      | -0.22                                | 0.563                    | N–H….O            | O              | GLN210 NE2        | 2.789                | ILE160; TRP166; GLN210; ARG167; TRP389; HIS393; SER153 |
| Dodecane                                  |                            |                                      |                          |                    |                |                   |                      | No interaction |
### Table 2 (continued)

| Compounds                        | Binding energy (kcal mol$^{-1}$) | Intermolecular efficiency (kcal mol$^{-1}$) | Inhibition constant (mM) | H-bond interaction Atom | Amino acid residue | Distance of D….A (Å) | Hydrophobic interaction |
|----------------------------------|----------------------------------|--------------------------------------------|--------------------------|--------------------------|-------------------|----------------------|------------------------|
| Dodecane, 1,1'-oxybis-           | -2.91                            | -0.12                                      | 7.41                     | -                        | -                 | -                    | ALA163; TRP166; ILE160; GLN210; SER153; GLY204; ASN248; HIS393; ASP390 |
| Hentriacontane                   | -3.48                            | -0.11                                      | 2.82                     | -                        | -                 | -                    | ALA163; ALA213; GLU208; TRP166; ILE160; GLN210; SER153; HIS393; ASP390; VAL391; GLN292; TYR368; SER364; SER365; TRP389 |
| Heptadecane                      | -4.18                            | -0.25                                      | 0.867                    | -                        | -                 | -                    | LEU176; GLN165; SER164; ALA163; ASN162; TRP166; GLN210; ILE160; SER153; HIS393; ARG167; TRP389 |
| Methyl dehydroabietate           | -6.46                            | -0.28                                      | 0.018                    | N–H….O                   | O                 | ALA163 N             | 2.989                  | ALA163; SER164; TRP166; ILE160; GLN210; TRP389; HIS393; ARG167; SER153 |
| Naphthalene, 6-chloro-1-nitro-    | -                                | -                                          | -                        | -                        | -                 | -                    | No interaction         |
| Octacosanol                      | -3.58                            | -0.12                                      | 2.38                     | N–H….O                   | O                 | SER164 N             | 2.834                  | ALA163; GLN165; SER164; LEU176; ILE210; GLU208; GLY204; ASN248; SER153; ARG167; HIS393; ASP390; TRP389 |
| Octadecane                       | -4.12                            | -0.23                                      | 0.961                    | -                        | -                 | -                    | -                      | ILE160; GLN210; SER153; ARG167; ASP390; TRP389; TRP166; ASN162; ALA163 |
| Phenol 2,4-bis(1,1-dimethylethyl) | -                                | -                                          | -                        | -                        | -                 | -                    | No interaction         |
| Phenol, 2-(1-phenylethyl)-        | -                                | -                                          | -                        | -                        | -                 | -                    | No interaction         |
| Phenol, 2,4-bis(1-phenylethyl)-   | -                                | -                                          | -                        | -                        | -                 | -                    | No interaction         |
| Phthalic acid, 2-ethylhexyl penty | -5.68                            | -0.23                                      | 0.069                    | -                        | -                 | -                    | ALA163; TRP166; ILE160; GLN210; ASP156; SER153; ARG167; HIS393; ASP390; SER364; TRP389 |
| Phthalic acid, butyl isohexyl pentyl ester | -5.23                            | -0.24                                      | 0.146                    | N–H….O                   | O                 | GLN210 NE2             | 2.969                  | ALA163; ASN162; ILE160; GLN210; SER153; TRP166; ARG167 |
| Phthalic acid, butyl isohexyl ester | -4.61                            | -0.19                                      | 0.416                    | N–H….O                   | O                 | ASN248 ND2             | 2.926                  | ILE160; TRP166; ARG167; ASP390; HIS393; ARG167; GLY204; GLU208; ASN248; ASN252; GLN210 |
| Silanetriamine, 1-azido-N,N,N’,N” | -3.23                            | -0.25                                      | 4.27                     | -                        | -                 | -                    | -                      | TRP166; TRP389; ASP390; HIS393; SER153; ARG167; GLN210 |
| Silanetriamine, 1-azido-N,N,N’,N” | -                                | -                                          | -                        | -                        | -                 | -                    | No interaction         |
| Tetradecane                      | -                                | -                                          | -                        | -                        | -                 | -                    | No interaction         |

*O* oxygen, *N* nitrogen, *H* hydrogen, *D* donor and, *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
treatment and extract alone tubs when compared to control due to the nature of extract color.

Effect of *U. fasciata* extracts against growth and virulence factors of *V. harveyi* during *P. monodon* larviculture

During the trial period, the growth of *V. harveyi* (Optical Density (OD) at 600 nm) was decreased as compared to control for all the sampling days. Further, the virulence factors of *V. harveyi* had also shown decreases by *U. fasciata* extract for all the sampling days as compared to the control (Table 5).

Statistical analysis

The results of growth and virulence factors of *V. harveyi* and values of CPM in *P. monodon* larviculture among *U. fasciata* extract treatment and control showed significances at \( p < 0.05 \) level by interpreting with statistical analysis.

Discussion

Molecular docking tools have played an essential role in the development of therapeutically vital small molecules, which follow either structure-based or ligand-based methods (Sliwoski et al. 2014). Due to the lack of an experimentally established crystal structure of a given protein, homology modeling has been applied to various areas of drug discovery including structure-related aspects of target validation, such as site-directed mutagenesis and drug ability assessment (Schmidt et al. 2014). Among the virulence factors of Vibrios, including *V. harveyi*, hemolysin can lyse RBC and other cells by producing pores on the cytoplasmic membrane (Wang et al. 2015).

During the docking investigations, the ligand molecules/inhibitors from *U. fasciata* abiding passed Lipinski’s rule of five, which showed its drug-likeness and possibility of its considerations for further pre-clinical studies. Lead optimization of the inhibitors from *U. fasciata* in the study was recognized by computation of drug-likeness properties and it was agreed about the report of Madeswaran et al. (2012) about the lead optimization of inhibitors by drug-likeness properties through computation. All the best-docked poses generated by each docking exhibited well-established bonds with one or more amino acids of SER153, HIS393, and ASP390 in the binding pocket of hemolysin protein. The top-ranked pose with the lowest docked-binding affinities and high docking scores is generally used as a standard selection in most of the docking programs (Azam et al. 2013). Likely, a lower docking score was showed a higher binding affinity known as inhibiting biological activity (Oda and Takahashi 2009).

The preliminary screening helps to compare the docking score of control molecule against ligands. Then the prescreened ligands were validated using AutoDock version
Table 3  Predicting drug-likeness of compounds of *U. fasciata* based on the Lipinski rule of five by SwissADME web tool

| 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 |
|-----------------------------------------------------|-----------------------------|-------------------------------------------|----------------------------------|--------------------------------------|--------------------------------------|
| 1,2-Benzenedicarboxylic acid, butyl                | 334.45                      | 4.93                                      | 1                                | 4                                    | 63.60                                |
| 1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester | 334.45                      | 5.09                                      | 0                                | 4                                    | 52.60                                |
| 1-Octadecene                                        | 252.48                      | 7.20                                      | 0                                | 0                                    | 88.17                                |
| 5-Eicosene, (E)-                                    | 280.53                      | 7.70                                      | 0                                | 0                                    | 97.78                                |
| 8-Heptadecene                                       | 238.45                      | 6.61                                      | 0                                | 0                                    | 83.36                                |
| Behenic alcohol                                     | 326.60                      | 7.61                                      | 1                                | 1                                    | 109.03                               |
| Bis(2-ethylhexyl) phthalate                         | 390.56                      | 6.10                                      | 0                                | 4                                    | 116.30                               |
| Dibutyl phthalate                                   | 278.34                      | 3.69                                      | 0                                | 4                                    | 77.84                                |
| Dodecane, 1,1’-oxybis-                              | 354.65                      | 8.46                                      | 0                                | 1                                    | 118.57                               |
| Hentriacontane                                       | 436.84                      | 11.90                                     | 0                                | 0                                    | 151.13                               |
| Heptadecane                                         | 240.47                      | 6.79                                      | 0                                | 0                                    | 83.83                                |
| Methyl dehydroabietate                              | 314.46                      | 4.82                                      | 0                                | 2                                    | 95.70                                |
| Octacosanol                                         | 410.76                      | 9.80                                      | 1                                | 1                                    | 137.87                               |
| Octadecane                                          | 254.49                      | 7.18                                      | 0                                | 0                                    | 88.64                                |
| Phthalic acid, 2-ethylhexyl pentyl ester            | 348.48                      | 5.32                                      | 0                                | 4                                    | 101.88                               |
| Phthalic acid, butyl isohexyl ester                 | 306.40                      | 4.40                                      | 0                                | 4                                    | 87.46                                |
| Phytol, acetate                                     | 338.57                      | 6.76                                      | 0                                | 2                                    | 108.68                               |
| Silanetriamine, 1-azido-N,N,N’,N”,N”-hexamethyl-    | 202.33                      | -0.03                                     | 0                                | 6                                    | 52.33                                |

Table 4  Evaluating of *U. fasciata* extract against *V. harveyi* in *P. monodon* postlarvae

| Parameters                                                                 | Days   | 0    | 5th | 10th | 15th | 20th | 25th | 30th |
|---------------------------------------------------------------------------|--------|------|-----|------|------|------|------|------|
| Cumulative percentage of mortality (CPM)                                   |        |      |     |      |      |      |      |      |
| Control (PL and *V. harveyi*)                                              |        | 0    | 13.66 ± 0.3 | 26.05 ± 0.9 | 35.63 ± 1.1 | 47.33 ± 1.5 | 62.13 ± 2.3 | 76.30 ± 2.9 |
| Treatment (PL, extract and *V. harveyi*)                                  |        | 0    | 6.96 ± 0.2 | 14.36 ± 0.3 | 21.33 ± 0.6 | 27.81 ± 1.1 | 36.63 ± 1.3 | 43.90 ± 1.3 |
| Reference Control (PL and extract only)                                    |        | 0    | 2.39 ± 0.1 | 6.19 ± 0.2 | 12.05 ± 0.5 | 18.13 ± 0.6 | 24.69 ± 0.9 | 29.56 ± 1.0 |
| Reference Control (PL only)                                                |        | 0    | 3.23 ± 0.1 | 6.03 ± 0.2 | 13.33 ± 0.5 | 17.43 ± 0.5 | 23.86 ± 1.0 | 28.39 ± 1.0 |
| Reference Control (PL, OTC and *V. harveyi*)                              |        | 0    | 7.91 ± 0.1 | 16.10 ± 0.3 | 23.66 ± 0.5 | 30.33 ± 1.3 | 39.11 ± 1.1 | 46.80 ± 1.1 |
| Treatment (CFU mL⁻¹)                                                       |        | 1.24 × 10⁶ | 2.42 × 10⁴ | 2.15 × 10⁴ | 7.40 × 10⁴ | 1.29 × 10⁴ | 9.40 × 10⁴ | 8.20 × 10⁴ |
| Total plate count                                                           |        | 1.15 × 10⁶ | 2.38 × 10⁴ | 1.56 × 10⁴ | 4.30 × 10³ | 3.40 × 10³ | 5.40 × 10³ | 4.10 × 10³ |
| *V. harveyi* count                                                         |        | 1.18 × 10⁶ | 3.18 × 10⁵ | 2.94 × 10⁵ | 1.51 × 10⁵ | 2.66 × 10⁴ | 1.80 × 10⁴ | 1.74 × 10⁴ |
| Control (CFU mL⁻¹)                                                         |        | 2.41 × 10⁶ | 3.40 × 10⁵ | 1.44 × 10⁵ | 1.45 × 10⁵ | 2.49 × 10⁴ | 1.74 × 10⁴ | 1.51 × 10⁵ |
| Total plate count                                                           |        | 1.81 ± 2  | 63.6 ± 3   | 127.5 ± 5  | 157.5 ± 5  | 197.9 ± 7  | 240.1 ± 9  | 266.5 ± 8 |
| V. *harveyi* count                                                         |        | 17.7 ± 3  | 60.9 ± 4   | 121.1 ± 4  | 156.3 ± 5  | 201.5 ± 9  | 236.9 ± 8  | 269.3 ± 9 |
| Average body weight of PL (mg)                                             |        | 18.1 ± 2  | 63.6 ± 3   | 127.5 ± 5  | 157.5 ± 5  | 197.9 ± 7  | 240.1 ± 9  | 266.5 ± 8 |
| Water quality parameters (treatment and control)                           |        | 29.0 ± 1.0 | 29.5 ± 1.0 | 29.0 ± 1.0 | 30.0 ± 1.0 | 30.0 ± 1.0 | 31.0 ± 1.0 | 30.0 ± 1.0 |
| Temperature (°C)                                                           |        | 20 ± 0.5  | 20 ± 0.5   | 20 ± 0.5   | 20 ± 0.5   | 21 ± 0.5   | 21 ± 0.5   | 21 ± 0.5   |
| Salinity (ppt)                                                             |        | 8.40 ± 0.2 | 8.50 ± 0.2 | 8.20 ± 0.2 | 8.40 ± 0.2 | 8.10 ± 0.2 | 8.40 ± 0.2 | 8.10 ± 0.2 |
| pH—Control                                                                 |        | 8.30 ± 0.2 | 8.40 ± 0.2 | 8.30 ± 0.2 | 8.50 ± 0.2 | 8.30 ± 0.2 | 8.20 ± 0.2 | 8.00 ± 0.2 |
| pH—Treatment                                                                |        | 1.18 ± 2  | 63.6 ± 3   | 127.5 ± 5  | 157.5 ± 5  | 197.9 ± 7  | 240.1 ± 9  | 266.5 ± 8 |

Values of average of three determinations with standard deviation
Growth and virulence factors production

Hemolytic activity

Cell surface hydrophobicity (SAT, M)

Table 5

Extract treatment against growth and virulence factors produced by V. harveyi and U. fasciata in LB broth

| Day  | Growth (OD 600 nm) | Phospholipase activity (OD 660 nm) | Lactase production (CPS) | Crude bacteriocin (OD 660 nm) | Protease production (CPS) | Luminescence production (CPS) | Luciferase production (CPS) | Phospholipase activity (OD 660 nm) |
|------|--------------------|-----------------------------------|-------------------------|-------------------------------|--------------------------|---------------------------|-----------------------------|-------------------------------|
| 5th  | 1.033 ± 0.03        | 0.778 ± 0.02                      | +                       | +                             | +                        | +                         | +                           | +                             |
| 10th | 1.482 ± 0.05        | 1.313 ± 0.01                      | +                       | +                             | +                        | +                         | +                           | +                             |
| 15th | 1.647 ± 0.05        | 1.393 ± 0.01                      | +                       | +                             | +                        | +                         | +                           | +                             |
| 20th | 1.357 ± 0.04        | 1.235 ± 0.01                      | +                       | +                             | +                        | +                         | +                           | +                             |
| 25th | 0.771 ± 0.01        | 0.615 ± 0.03                      | +                       | +                             | +                        | +                         | +                           | +                             |
| 30th | 1.152 ± 0.06        | 1.065 ± 0.04                      | +                       | +                             | +                        | +                         | +                           | +                             |

+ non-existent, weak, + moderate, ++ high, +++ very high; SAT (>4.0 M) strongly hydrophobic, 1.0–2.0 M moderately hydrophobic, 0.0–1.0 Molarity (M) not hydrophobic.
the counting of V. harveyi has been reduced in growth and virulence factors against U. fasciata extract. Davies and Marques (2009) have shown that saturated and unsaturated fatty acids can prevent initial bacterial adhesion, subsequent biofilm formation, and later induce lysis of bacterial protoplasts.

The antioxidant and anti-hemolytic activity of many Ulva species was found with momentous results (Farasat et al. 2014). Recently, anti-hemolytic activity exhibited by coriander leaves (Coriandrum sativum L.) and filamentous green alga Enteromorpha intestinalis was reported against H2O2-induced hemolytic activity (Soltani et al. 2012). Hereafter, it was evident that the anti-hemolytic activity of coriander and filamentous green alga was caused due to the compounds quenched H2O2 before it attacked the biomolecules of erythrocyte membrane to cause oxidative hemolysis on the effects of green tea as reported by Costa et al. (2009). Further, diterpene isolated from marine brown alga Canistrocarpus cervicornis caused its anti-hemolytic activity, as well as fibrinogen or plasma clotting induced by Lachesis muta snake venom protein, was reported (Moura et al. 2011). The inhibiting activity on snake venom protein by brown alga was compared with the antagonism of U. fasciata inhibitors against hemolysin protein. Moreover, in the P. monodon larviculture trials, an extract of U. fasciata exhibited a lowest (non-existent) level of inhibition or virulence of hemolysis activity of V. harveyi on sheep blood agar than control including reductions of growth and other virulence factors.

**Conclusion**

The results indicated that the inhibitors exhibited better docking scores and showed higher binding affinity, followed no violation of the rule by the ligands used in docking for determining drug pharmacological activity. Also found, anti-hemolytic activity, reductions in the growth and virulence factors of V. harveyi by U. fasciata extract during the experiment. In the larviculture trial, U. fasciata showed improved survival level on P. monodon postlarvae than control. Hence, the present findings indicated that the marine green alga U. fasciata may be a remarkable source for developing potent bio-inhibitors in shrimp disease management.

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**Author contributions** KS and SK designed research and wrote the manuscript. KS and BV performed docking work and analyzed data. BV, KPI, SB and AP 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.

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