International Journal of Peptide Research and Therapeutics (2019) 25:591–604
https://doi.org/10.1007/s10989-018-9705-x

Sea Anemones as Potential Source for Bioactive Metabolites

S. Thangaraj[1] · **S. Bragadeeswaran**[2] · **V. Gokula**[1]

Accepted: 4 April 2018 / Published online: 13 April 2018

Abstract
Marine organisms are novel sources for biologically active compounds which are potentially valuable materials in biomedical research. In the present investigation, the potential bioactive compounds were isolated from the sea anemone *Heteractis aurora* collected from Mandapam, Southeast coast India. The maximum inhibition zone was found against bacterial pathogens (*Klebsiella oxytoca* 7.2 ± 1.5 and *Escherichia coli* 8.1 ± 0.2) followed by fungal pathogens (*Botrytis cinerea* 5.3 ± 0.5 and *Trichoderma koning* 4.2 ± 1.2). The antioxidant activity was found to be 42.2 ± 1.14%, whereas hemolytic activity was recorded as 64 Hemolytic unit against chicken blood erythrocytes. The chemical characterizations of sea anemone extract were carried out by FT-IR, GC–MS and NMR (^{13}C, ^{1}H) spectroscopy. The FT-IR results showed the presence of phenyl ring: C–CH_3 and C=C stretching. GC–MS analysis revealed the presence of acetic acid-17-acetoxy-4,4,10,13-tetramethyl-7-oxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta (a) phenanthren-3-yl (ester) and 4[-4-diethylamino-1-methylbutylamino]-1,2 dimethoxy-6-bromonaphthalene. These identified compounds were subjected for molecular docking analysis against the target protein enoyl-acyl carrier protein reductase which revealed that acetic acid-17-acetoxy-4,4,10,13-tetramethyl-7-oxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta (a) phenanthren-3-yl showed better docking interaction than the commercial standard drug Tryptanthrine.

Keywords Sea anemone · *Heteractis aurora* · Hemolytic unit · *Klebsiella oxytoca* · *Trichoderma koning* and Tryptanthrine

Introduction

Marine organisms are the novel sources of biologically active compounds and considered as very productive field, which has led to the discovery of various novel pharmacological medicines (Faulkner 2001). Cnidarian venoms are potentially valuable materials for biomedical research and drug development (Bragadeeswaran et al. 2011). This phylum provides a large number of natural products, including proteins and secondary metabolites with either toxic or biomedical properties (Rojas et al. 2002; Thangaraj and Bragadeeswaran 2012). Sea anemone has neurotoxic effect that paralyses small marine animals with nematocysts in the tentacles. This mechanism is one of the fastest actions in the animal kingdom (Patton 1995). Several methods exist for isolation of nematocysts from cnidarian tissues; most of them are tedious. Chemical, biochemical and pharmacological investigations of this phylum have mainly been focused on members of the classes Alcyonaria: soft corals and gorgonians (Ospina and Rodriguez 2006; Chen et al. 2012), Zooantharia (anemones) (Bragadeeswaran et al. 2011; Thangaraj and Bragadeeswaran 2012) and Cubozoa (Jellyfishes) (Rojas et al. 2002; Suganthi et al. 2011).

The toxins from several species of sea anemone have different actions of paralysis on crabs (Thangaraj and Bragadeeswaran 2012) and mammals (Beress and Beress 1971). The cytolytic and lethal effects of equinatoxin have been reported from sea anemone, *Actinia equine* (Ferlan and Lebez 1974; Ferlan and Levez 1976) and antimicrobial activity against several human pathogens (Thangaraj et al. 2011). Generally, the sea anemones produce two types of proteinaceous toxins: neurotoxins, act mainly on the ion channels (Honma and Shiomi 2005), and cytolysins or actinoporins, show lytic activity on a variety of cells (Anderluh and Macek 2002; Alvarez et al. 2009; Kristan et al. 2009).

✉ S. Thangaraj
thangamjayam@gmail.com

[1] PG & Research Department of Zoology, National College, Trichy 620001, Tamil Nadu, India

[2] CAS in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai, Tamil Nadu 608502, India

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* is one of the leading cause of mortality, indicated by more than nine million cases of TB in 2009 (World Health Organization 2010). *M. tuberculosis* has two features that render it the deadliest infectious disease to date, its high infectivity (virulence) and its ability to enter latency for subsequent reactivation, a phenomenon that leads to a deadly synergy with AIDS (Bates et al. 2004; Takayama et al. 2005; Lin et al. 2005). Current standard treatment regimen of TB is severely hampered by multidrug resistant tuberculosis (MDR-TB), extensively drug-resistant tuberculosis (XDR-TB) and HIV co-infection with TB (WHO 2010). This fact prompts the researchers to develop novel and more potent drugs for TB. Enoyl-acyl carrier protein reductase (or ENR) (EC 1.3.1.9) is a key enzyme of the type II fatty acid synthesis (FAS) system (Kapoor et al. 2004). ENR is a promising target for narrow-spectrum antibacterial drug discovery because of its potential role in metabolism and its conserved sequence across bacterial species. Moreover, the bacterial ENR sequence and structural organization are distinctly different from those of mammalian fatty acid biosynthesis enzymes (Ling et al. 2004). The elongation module of fatty acid biosynthesis consists of four iterative steps: decarboxylative condensation, NADPH dependent reduction, dehydration and NADH-dependent reduction (Rock and Cronan 1996). The fourth step of NADH-dependent reduction is carried out by enoyl-acyl carrier protein (ACP) reductase (ENR), which reduces the trans-2 enoyl bond of enoyl-ACP substrates to saturated acyl-ACPs (Smith et al. 2003).

Molecular docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Knowledge of the preferred orientation in turn may be used to predict the strength of the association or binding affinity between two molecules using for example scoring functions (Ewing et al. 2001; Bursulaya et al. 2003). Molecular docking has attracted increasing attention as a way to predict the geometries of bimolecular complexes (Kuntz et al. 1994). After several decades of neglect, tuberculosis is receiving the increased attention that this global public health problem deserves. Although most of these new resources are being appropriately invested in TB control programs in countries where the TB epidemic is most severe, a significant commitment also is being made to basic research and the development of new diagnostic, treatment and prevention tools, including new TB drugs (Zhang et al. 2003). Hence the present work was carried out for the characterization of bioactive metabolites from the sea anemones and their antimicrobial and antioxidant activities followed by molecular docking analysis to investigate the binding of sea anemone derived compounds on the active site of *M. tuberculosis* enoyl-acyl carrier protein (ACP) reductase (InhA) in an attempt to address the mycobacterial resistance against various drugs.

Materials and Methods

Sample Collection

The toxin was extracted from sea anemones *Heteractis aurora, Heteractis crispa* and *Stichodactyla haddoni* collected from the Gulf of Mannar (8°47′–9°15′N Latitude and 78°12′–79°14′E Longitude), Southeast coast of Tamil Nadu, India. Sea anemone samples were kept in a polythene bag with seawater, air and transported to the laboratory, Faculty of Marine Science, Parangipettai.

Extraction of Metabolites from Sea Anemones

Bioactive metabolites were extracted from sea anemones nematocyst by following the method described by Kem et al. (1989). For the extraction of the secondary metabolites in sea anemones, animals were washed thoroughly in distilled water to remove the impurities. Then the washed specimens were placed in separate glass bowl containing 400 mL of distilled water and frozen at − 40 °C for 30 min. The extractions were repeated for three times, after this process the animals were removed from the distilled water, then the distilled water solution was centrifuged at 4000 rpm for 10 min at 4 °C. After centrifugation, the supernatant was collected for the further purification using Thin Layer Chromatography (Buckley et al. 1975) and the purified compound was stored at − 4 °C for further analysis.

Antimicrobial Activity

Effects of the sea anemone derived crude extracts on antimicrobial activity were assessed by agar disc diffusion method (Brumfitt et al. 1990; Galeano and Martinez 2007). The antimicrobial activity was tested against a total of 20 clinical pathogens (10 bacteria: *Staphylococcus aureus, Streptococcus pyogenes, Vibrio cholera, Vibrio parahaemolyticus, Klebsiella pneumonia, Klebsiella oxytoca, Salmonella typhi, Salmonella paratyphi, Escherichia coli* and *Proteus mirabilis*, and ten fungal strains: *Aspergillus niger, Botrytis cinerea, Cladosporium cucumerinum, Penicillium expansum, Rhizopus oryzae, Trichoderma harzianum, Trichoderma koningi, Aspergillus fumigates, Pneumocystis jirovecii, Stachybotrys chartarum)* obtained from Raja Muthaiya Medical College and Microbiology culture collection centre, Annamalai University.

Agar Disc Diffusion Method

A loop full of fresh cultures of bacterial strains were inoculated into nutrient broth medium and incubated at 37 °C for

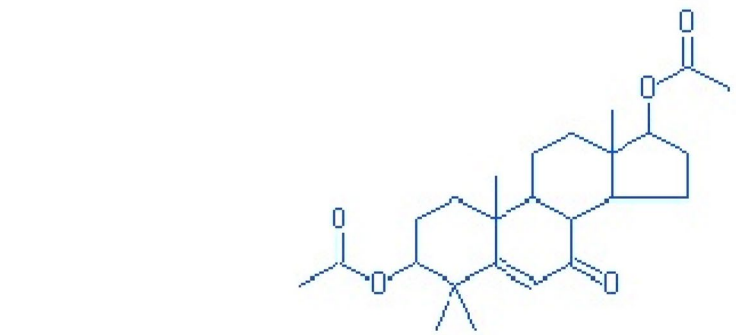
24 h, while fungal strains were inoculated into sabouraud's broth medium and incubated at 28 °C for 48 h. 100 µL of cell suspension for each of the bacterial and fungal strains were collected and poured onto the Muller Hinton Agar and potato dextrose Agar plates respectively. Cultures were spread on the plates using a glass spreader. Serile discs of 6 mm width were impregnated with 25 µL of crude extracts of *Heteractis aurora, Heteractis crispa* and *Stichodactyla haddoni*. The plates were incubated at 37 °C for 24 h for bacteria and 28 °C at 48 h for fungi. The diameter of inhibition zones (in millimeters) around the discs was measured after 24 h for bacteria and 96 h for fungal pathogens. Among the three sea anemones species (*H. aurora, H. crispa* and *S. haddoni)*, the extract of *H. aurora* alone showed pronounced inhibition effect against bacterial and fungal pathogens. Hence, the extract of *H. aurora* alone was used for further screening (antioxidant assays, hemolytic assay, compound identification and molecular docking analysis) in the present study.

Antioxidant Assays

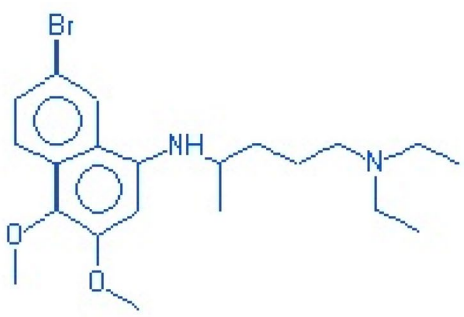
The antioxidant property of potent crude extract of *H. aurora* was determined for the present study by using five different assays: Total antioxidant capacity (Prieto et al. 1999); Total phenol content using the Folin–Ciocalteu reagent (Singleton et al. 1999); Radical scavenging activity using the DPPH (1,2-diphenyl-1-picrylhydrazyl) free radical (Duan et al. 2006); Hydrogen peroxide radical inhibition activity (Gulcin et al. 2005) and Reducing power (Oyaizu 1986), whereas Ascorbic acid and Gallic acid were used as a standard.

Hemolytic Assay

The hemolytic activity of the extract of *H. aurora* was tested on chicken, goat, cow and human erythrocytes following Thangaraj and Bragadeeswaran (2012). This assay was performed on a 'V' shaped sterile Laxbro microtitre plate (India). Serial twofold dilutions of the sea anemone extract (100 µL; 1 mg crude in 1 mL PBS) were made in PBS (pH 7.2) starting from 1:2. An equal volume of 1% human RBC was added to each well. The plate was shaken to mix the RBC and sea anemone extract. The plates were incubated at room temperature for 2 h before taking the reading. Erythrocyte suspensions and distilled water was added (100 µL respectively) served as blanks for negative control. Button formation at the bottom of the wells was taken as negative. The reciprocal of the highest dilution of the venom extracted showing the hemolysis was defined as one hemolytic unit.



Acetic acid-17-acetoxy-4,4,10,13-tetramethyl-7-oxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta(a)phenanthren-3-yl (ester)



4[-4-Diethylamino-1-methylbutylamino]-1,2-dimethoxy-6-bromonaphthalene

Fig. 1 Sea anemone, *H. aurora* derived active compounds identified through GC–MS

Table 1 Antimicrobial activity of sea anemones extract against bacterial and fungal pathogens

Crude extract of sea anemone	Anti-bacterial activity										
	Zone of inhibition against bacterial pathogens (mm)										
	Staphylococcus aureus	*Streptococcus pyogenes*	*Vibrio cholerae*	*Vibrio parahaemolyticus*	*Klebsiella pneumoniae*	*Klebsiella oxytoca*	*Salmonella typhi*	*Salmonella paratyphi*	*Escherichia coli*	*Proteus mirabilis*	Average (mm)
H. aurora	5.2 ± 0.2	0.2 ± 0.1	3.2 ± 0.2	5.1 ± 0.5	4.1 ± 0.2	7.2 ± 1.5	6.1 ± 1.2	5.1 ± 0.6	8.1 ± 0.2	4.6 ± 1.2	4.89 ± 1.3
H. crispa	2.6 ± 0.1	0	1.3 ± 0.6	2.3 ± 0.6	2.2 ± 0.3	1.3 ± 0.1	2.3 ± 1.2	3.2 ± 0.2	5.2 ± 0.3	2.4 ± 1.2	2.28 ± 0.3
S. haddoni	1.1 ± 0.3	1.1 ± 0.3	0	1.1 ± 0.2	2.3 ± 0.5	2.1 ± 0.3	1.1 ± 0.6	5.1 ± 1.2	1.1 ± 0.4	1.5 ± 1.2	1.65 ± 0.5

Crude extract of sea anemone	Antifungal activity										
	Aspergillus niger	*Botrytis cinerea*	*Cladosporium cucumerinum*	*Penicillium expansum*	*Rhizopus oryzae*	*Trichoderma harzianum*	*Trichoderma koningi*	*Aspergillus fumigatus*	*Pneumocystis jirovecii*	*Stachybotrys chartarum*	Average (mm)
H. aurora	3.1 ± 1.2	5.3 ± 0.5	3.2 ± 0.2	1.1 ± 0.2	2.2 ± 1.2	1.3 ± 0.6	4.2 ± 1.2	0	0	0	2.04
H. crispa	1.2 ± 0.3	2.1 ± 0.6	5.1 ± 0.6	3.2 ± 1.5	1.1 ± 0.6	2.2 ± 1.5	1.1 ± 0.6	0	0	1.3 ± 1.5	1.73
S. haddoni	0	1.2 ± 0.6	3.3 ± 0.5	1.2 ± 0.2	0	1.1 ± 0.6	1.2 ± 0.3	1.3 ± 0.6	0	0	0.93

Spectroscopy and FT-IR Spectrum

The chemical configuration of bioactive compound in the crude extract of *H. aurora* was analyzed by UV–Visible, Fourier Transform Infrared Spectroscopy (4000–500 cm^{-1} range), Gas Chromatography–Mass Spectroscopy (GC–MS) and Nuclear Magnetic Resonances (NMR) spectroscopy. UV–Visible spectrum was obtained using a Perkin Elmer (Model: Lambda 25; Serial Number: 501309025) double beam spectrophotometer.

Identification of Compounds

The interpretation of mass spectrum GC–MS was conducted using the database of National Institute Standard and Technique (NIST08s), VR2.0 and FAME. The spectrum of the unknown component was compared with the spectrum of the known component stored in the NIST08s, VR2.0 and FAME library. The Name, Molecular weight, Molecular formula and Structure of the component of the test material was determined.

Molecular Docking Analysis

The crystal structure of target protein enoyl-acyl carrier protein reductase (PDB ID: 2H7I), having the resolution of 1.80 Å was retrieved from the protein data bank (PDB) (http://www.rcsb.org/pdb). All water molecules were removed and hydrogen atoms were added to the target protein molecule. The compounds identified through GC–MS analysis were taken as ligand molecule (Fig. 1). Tryptanthrine (CID: 73549) was considered as a control ligand. ChemSketch, chemically intelligent drawing interface freeware developed by Advanced Chemistry Development, Inc., (http://www.acdlabs.com) was used to construct the structure of the ligands. The chemical structures were generated from SMILES notation (Simplified Molecular Input Line Entry Specification) by using the Chemsketch Software (http://www.acdlabs.com) and then saved in ".mol" file.

"Active site prediction tool" from SCFBio Server (http://www.scfbio-iitd.res.in/dock/ActiveSite.jsp) was used to predict active site of the target protein. The details on the total number of active sites along with information on their amino acid sequence, cavity points and the average volume of the cavity were also obtained by using tool. To find the reasonable binding geometries and explore the protein ligand interactions, Argus Lab 4.0.1, most common and freely available software was used for docking analysis (Planaria Software LLC, Seattle, WA, USA, http://www.arguslab.com). The selected residues of the receptor were defined to be a part of the binding site. The inhibitor and target protein were geometrically optimized and "GA dock" docking engine was used. Calculation type was set to "Dock" mode whereas "flexible mode" was selected for the ligand. Grid resolution was set to 0.40 Å (Sahu et al. 2012). Least energy represented the easy binding character of ligand and receptor. Hence, the docking poses saved for each compound were ranked according to their dock score function.

After docking, the docked structure was saved as "pdb" file and further interactions study was carried out in Pymol visualization tool (http://www.pymol.org) and the binding sites were predict using "Discovery Studio v3.1" software. The predicted binding sites, based on the binding energy and amino acids make up the binding cavity. Here ligand binding site represents the site where the ligands most efficiently bind with the protein, among all the active site. Ligand property was predicted by using "Lipinski drug Filters" (http://www.scfbio-iitd.res.in/utility/LipinskiFilters.jsp).

Results

Antimicrobial Activity

Sea anemone extracts showed pronounced antimicrobial activity against the tested pathogens with the exception of one bacterial (*Streptococcus pyogenes)* and three fungal pathogens (*Pneumocystis jirovecii, Aspergillus fumigatus* and *Stachybotrys chartarum*) (Table 1).

Antioxidant Activity

The extract of *H. aurora* showed prominent activities in all the five assays where the total antioxidant was recorded as 0.08 ± 0.01 mg g^{-1}, total phenol (0.09 ± 0.02 mg g^{-1}), DPPH radical scavenging activity ($42.2 \pm 1.14\%$), reducing power (0.27 ± 0.01 mg g^{-1}) and hydrogen peroxide radical scavenging activity was found to be $41.86 \pm 1.14\%$.

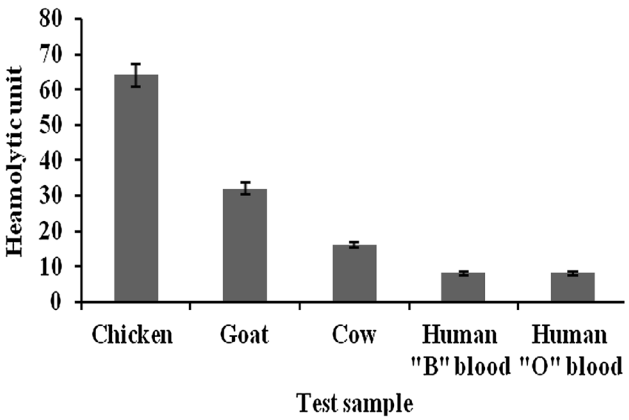


Fig. 2 Hemolytic effect of *H. aurora* extract on various blood groups

Hemolytic Activity

Hemolytic effect of the *H. aurora* derived crude extract was tested against various RBC cells such as chicken, goat, cow and human blood (Fig. 2). Among the five different RBC blood cells, chicken blood exhibited the maximum hemolytic activity of 64 Hemolytic Unit (HU) followed by goat (32 HU), Cow (16HU), and human blood group (8HU).

Table 2 FT-IR analysis of sea anemone, *H. aurora* extract

S. no	Frequency, cm^{-1}	Bond	Functional group
1	648.08 (m)	C–Br stretch	Alkyl halides
2	835.18 (m)	C–Cl stretch	Alkyl halides
3	900.76 (s, b)	N–H wag	1°, 2° amines
4	962.48 (s)	=C–H bend	Alkenes
5	1199.72 (m)	C–N stretch	Aliphatic amines
6	1321.24 (s)	C–N stretch	Aromatic amines
7	1452.40 (m)	C–H bend	Alkanes
8	1506.41 (s)	N–O asymmetric stretch	Nitro compounds
9	1581.63 (m)	N–H bend	1° amines
10	1643.35 (m)	–C=C– stretch	Alkenes
11	1691.57 (s)	C=O stretch	α, β unsaturated aldehydes, ketones
12	2144.84 (w)	–C≡C– stretch	Alkynes
13	2198.85	–C≡C– stretch	Alkynes
14	2949.16 (m)	C–H stretch alkanes	C–H stretch alkanes
15	3379.29 (m)	N–H stretch	1°, 2° amines, amides

s, b strong, *b* broad, *m* medium, *w* weak, *b, s* broad, *s* strong

Chemical Characterizations of the Sea Anemone Derived Compounds

FT-IR results showed that the phenyl ring and C=C stretching frequency appeared at 3604 and 1643 cm^{-1}, $C–CH_3$ stretching frequency appeared at 2981 cm^{-1} (Table 2; Fig. 3). Whereas CH_3 and CH_2 deformation frequency showed at 1383 and 1444 cm^{-1}. GC–MS analysis revealed that the crude extract of *H. aurora* has the following compounds; acetic acid-17-acetoxy-4,4,10,13-tetramethyl-7-oxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta (a) phenanthren-3-yl (ester) and 4[-4-diethylamino-1-methylbutylamino]-1,2 dimethoxy-6-bromonaphthalene at the retention times of 13.71, 15.63 (Fig. 4). NMR results revealed the presences of following functional groups; $C–CH_3$ bond observed at 1.02 chemical shift, whereas CH_2 and Cyclohexane ring –H found at chemical shift of 2.59, 7.12 respectively (Fig. 5).

Discussion

Marine environment is a potential source for the extraction and identification of novel bioactive metabolites (Battison et al. 2008; Tadesse et al. 2008). The present results showed that the *H. aurora* derived crude extract had the

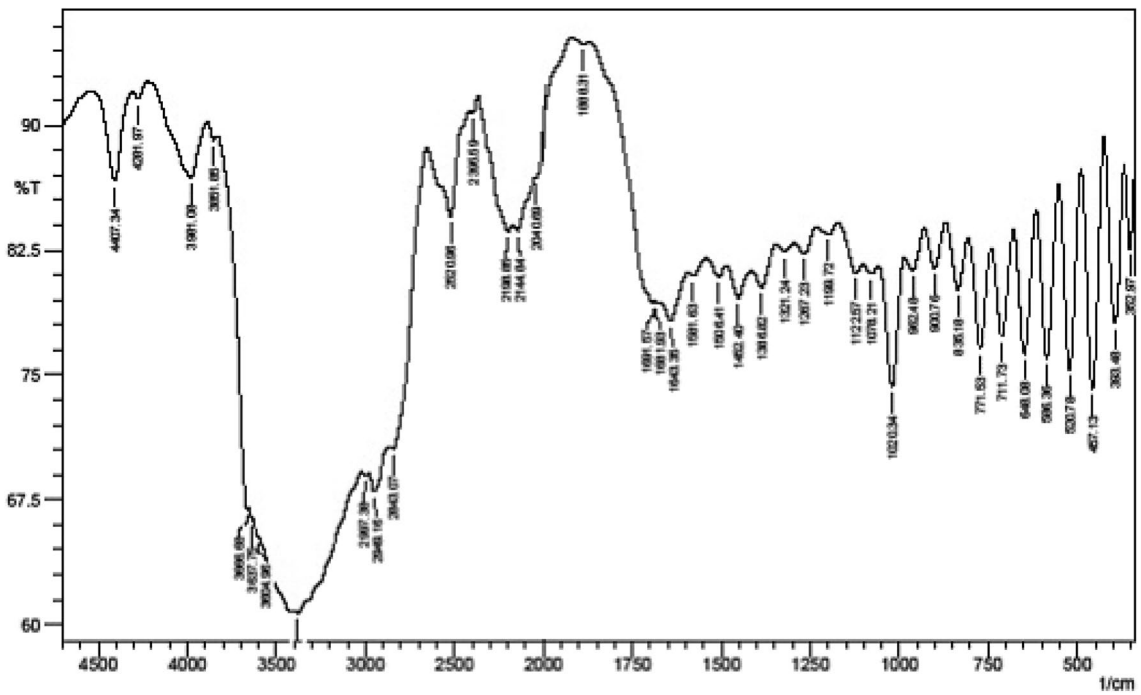


Fig. 3 FT-IR chromatogram of sea anemone, *H. aurora* extract

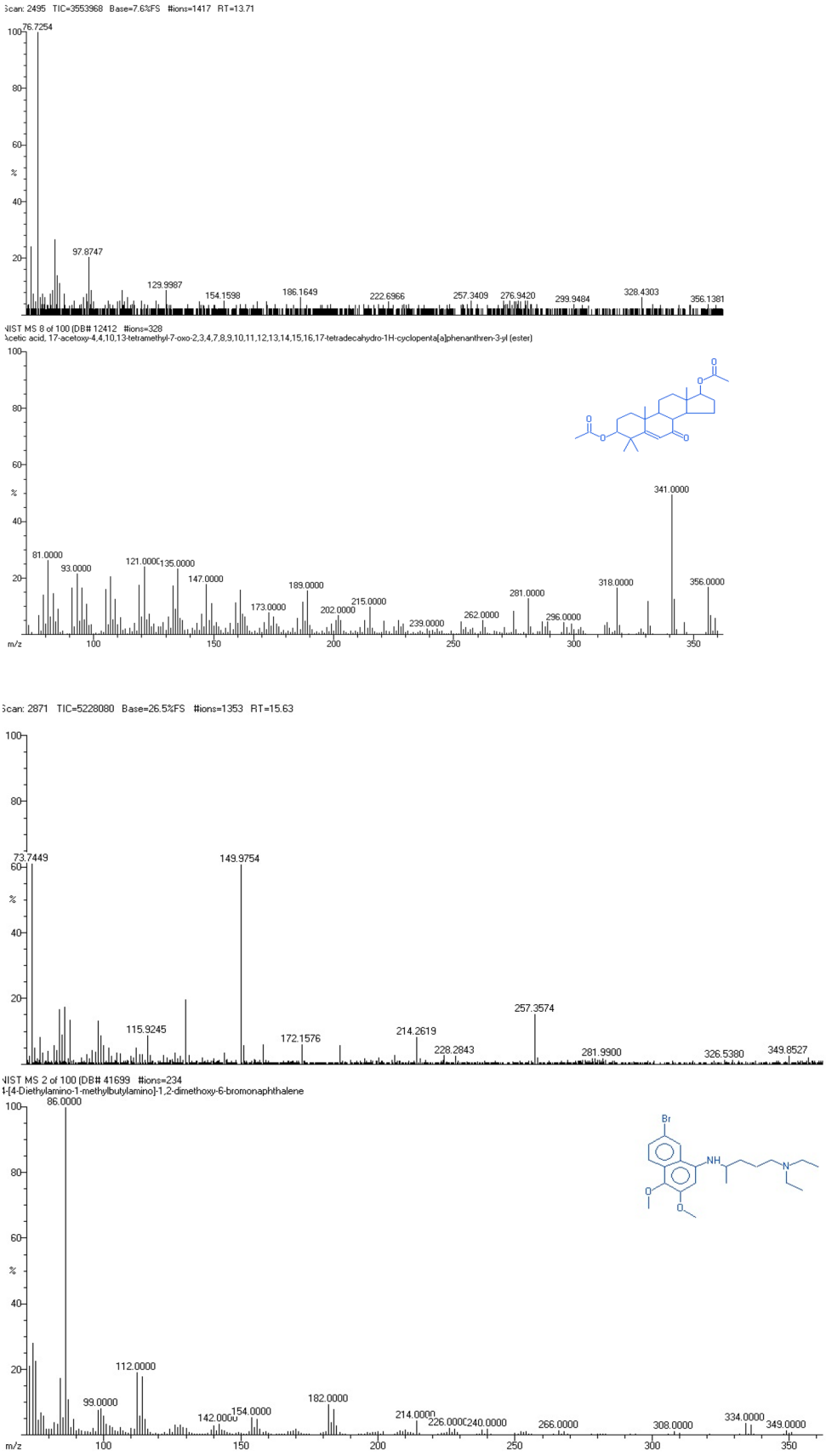


Fig. 4 GC–MS chromatogram of sea anemone, *H. aurora* extract

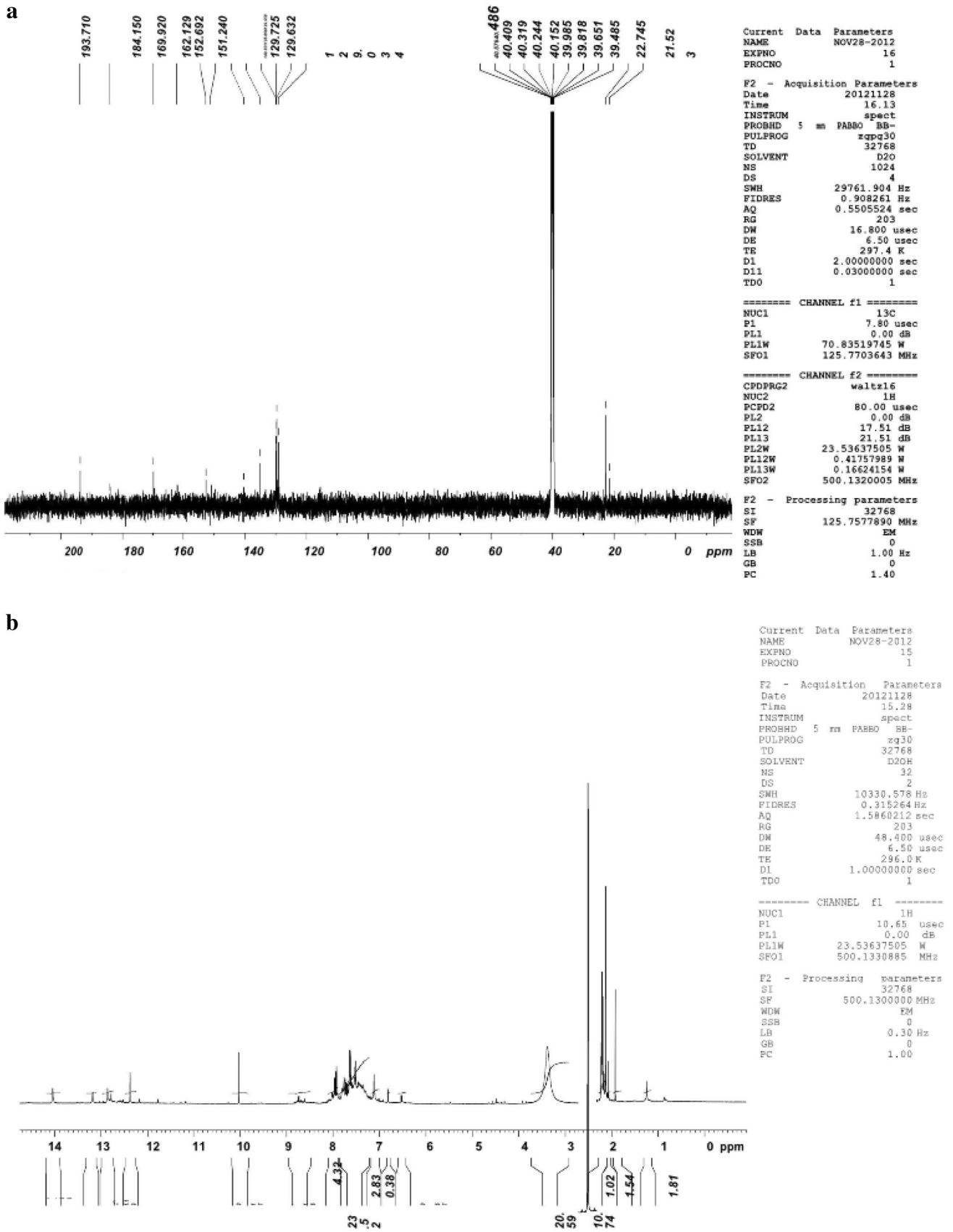


Fig. 5 Structural elucidation of *H. aurora* derived compound by NMR spectroscopy **a** ^{13}C NMR; **b** ^{1}H NMR

highest antibacterial effects against *Klebsiella oxytoca* and *E. coli* pathogens as reported elsewhere for sea anemones (Wei et al. 2007; Williams et al. 2007; Thangaraj et al. 2011). Among the Phylum Cnidarians, antioxidant property was well established in jelly fish and corals (Griffin and Bhagooli 2004; Yu et al. 2006, 2007; Balamurugan and Menon 2009). For the first time, in the present study, this property was also established for *H. aurora*.

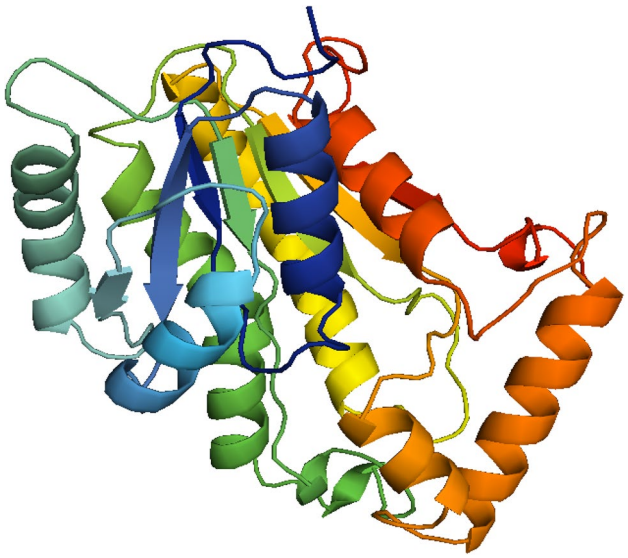


Fig. 6 3D crystal structure of enoyl-acyl carrier protein reductase

The present study revealed that the sea anemone derived crude extract acts as a potent source for antimicrobial, antioxidant and hemolytic activity as suggested by Jensen et al. 1996; Roussis et al. 2001(in marine invertebrates), Encarnacion et al. 2000; Koh et al. 2002; Harder et al. 2003; Marquis et al. 2005, Bala et al. 1999; Wilsanand et al. 1999; Geffen and Rosenberg 2005 (in scleractinian gorgonian and soft corals). Besides, Mamelona et al. (2007) studied the phenolic contents and antioxidant capacity in the various parts such as digestive tract, gonads, muscles and respiratory apparatus from the sea cucumber, *Cucumaria frondosa*. The coelomic fluid of some sea cucumber such as *Bohadschia marmorata vitiensis, Stichopus variegatus* and *S. badionotus* showed the potent antioxidant activities (Hawa et al. 1999).

The maximum hemolytic activity was observed against chicken blood (64 HU) as reported by Thangaraj and Bragadeeswaran (2012) for *S. mertensii* and *S. gigantean*.

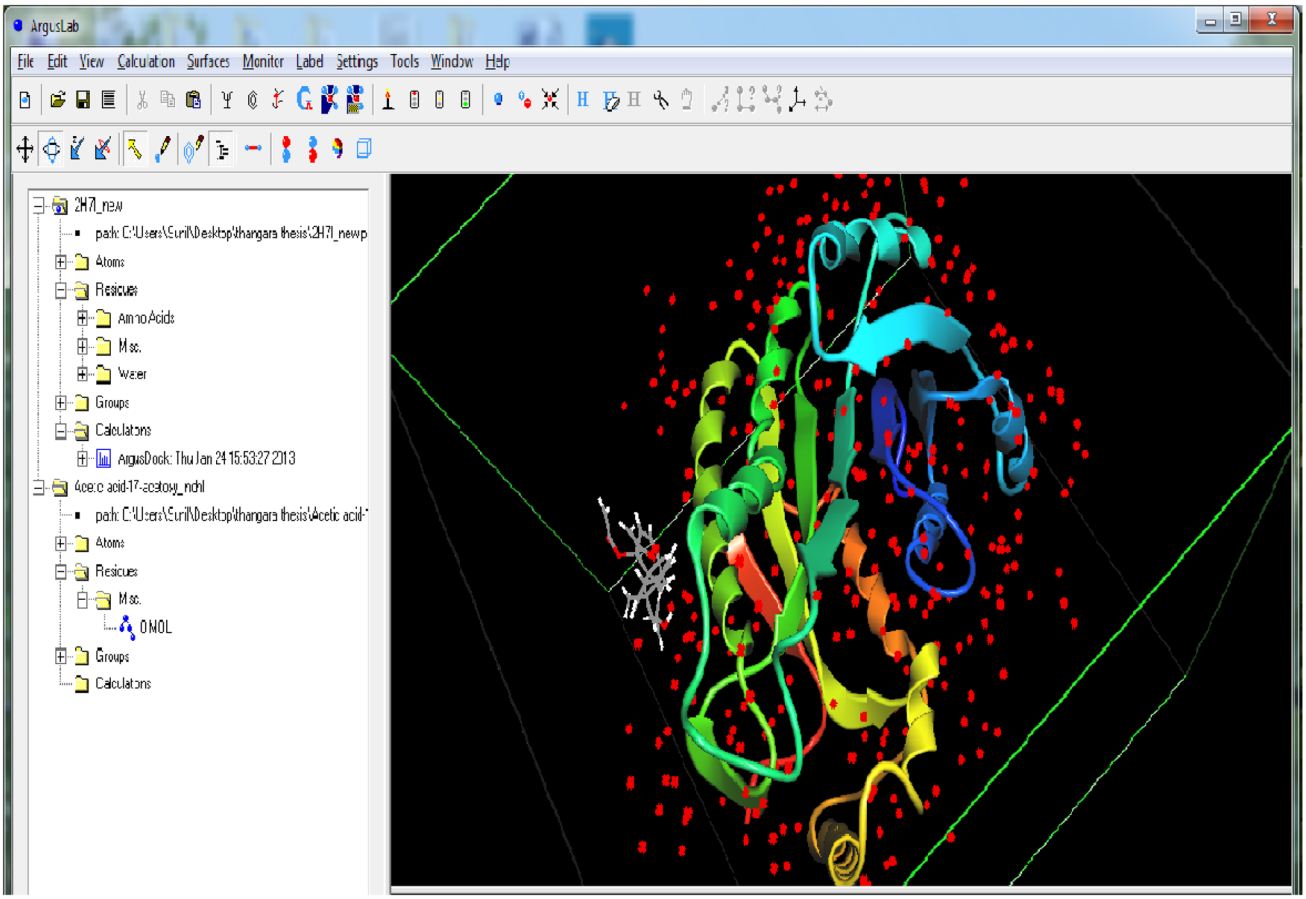


Fig. 7 Screenshot of the molecular docking process in Arguslab software

Table 3 Docking results of sea anemone derived compounds against enoyl-acyl carrier protein reductase

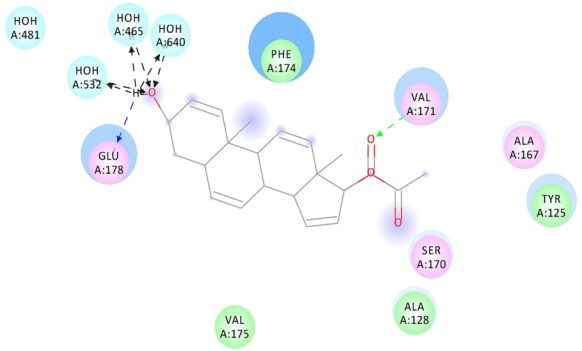
Compound name	Reference/source	Molecular weight (g/mol)	Hydrogen donor/acceptor	Docking energy level (kcal/mol)
Acetic acid-17-acetoxy-4,4,10,13-tetramethyl-7-oxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta (a) phenanthren-3-yl	Novel, first time reported in the present study	374.52	(1, 3)	− 17.932
4[-4-Diethylamino-1-methylbutylamino]-1,2 dimethoxy-6-bromonaphthalene	CID: 559477	423.38	(1, 4)	− 13.285
Tryptanthrine	CID: 73549	248.236	(0, 3)	− 7.351

However, Santamaria et al. (2002) and Kohno et al. (2009) reported the higher hemolytic effects in *Anthopleura asiatica* and *Bartholomea annulata* against mammalian erythrocytes. Similarly jelly fishes *Carybdea marsupialis, Rhopilema esculentum* and *Cassiopea xamachana* also showed such have potential hemolytic effects against some mammalian blood cells (Rottini et al. 1995; Torres et al. 2001; Yu et al. 2007; Jinhua et al. 2009).

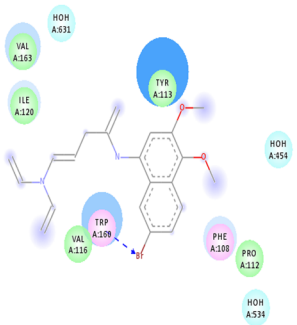
Microbial infections are a growing problem in contemporary medicine, yet only a few antimicrobial agents are used in clinical practice. *M. tuberculosis* is a pathogenic bacterial species in the genus *Mycobacterium* and the causative agent of most cases of tuberculosis (Ryan and Ray 2004). In the present study, to understand the interactions between the novel compounds identified from the sea anemone, *Heteractis aurora* and target protein (enoyl-acyl carrier protein reductase) (Fig. 6) and to explore their binding mode, docking study was performed using ArgusLab 4.0.1 (Fig. 7). The docked ligand molecules were selected based on docking energy and good interaction with the active site residues and the results are shown in Table 3 and Fig. 8. InhA has been identified as the primary target of isoniazid (INH), one of the most effective first-line anti-TB drugs (Rozwarski et al. 1998; Vilcheze et al. 2006). Both the ligand molecules (1) acetic acid-17-acetoxy 4,4,10,13-tetramethyl-7-oxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H cyclopenta (a) phenanthren-3-yl (ester) and (2) 4[-4-diethylamino-1-methylbutylamino]-1,2 dimethoxy-6-bromonaphthalene showed better docking score − 17.932 and − 13.285 kcal/mol respectively than the standard drug Tryptanthrine (− 7.351 kcal/mol). Acetic acid and its derivative have been well reported for its potent antibacterial activity (Ali and Shaharyar 2007). Moreover, structure-based drug design has also been reported against InhA (Freundlich et al. 2009). They have used a series of triclosan derivatives with modifications at the 5-chloro of triclosan, 5-substituted triclosan derivatives against the target protein. Antibacterial activity of *Heteractis* sp. has been documented against aquatic bacterial and clinical pathogens (Devi et al. 2012). Molecular docking has been a powerful tool for medicinal chemists, allowing the rapid and inexpensive identification of a pool of potential protein inhibitors (Jorgensen 2004; Brooijmans and Kuntz 2003). This is the first report of sea anemone derived compound against the enoyl-acyl carrier protein reductase. In the present study, totally 18 cavities were predicted by the active site prediction tool. Pymol & discovery studies software were used for molecular visualization (Fig. 9). Both the identified compounds passed the Lipinski rule as evident in the Table 3. Thus, the in silico docking analysis revealed that potential of sea anemone derived compounds as the novel inhibitor of enoyl-acyl carrier protein reductase (Fig. 6).

Conclusions

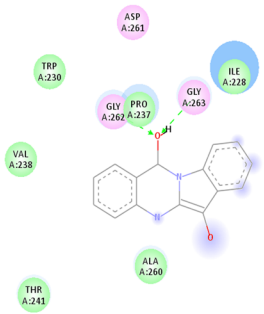
This study showed that extract prepared from sea anemone showed high antibacterial activity. It also showed the existence of haemolytic factors in the hemolytic activity. However, it is unknown whether the same factor is responsible for both antibacterial and haemolytic activities or not. Further purification of the active compounds is necessary in order to identify their chemical nature and to evaluate their potential as novel drugs. Molecular docking further substantiated that enoyl-acyl carrier protein reductase is an alternative target for development of anti-virulence drug lead compounds. Enoyl-acyl carrier protein reductase synthesis pathway inhibitors could be used synergistically with conventional antibiotics to treat *M. tuberculosis*. Among the sea anemone, *H. aurora* derived compounds, Acetic acid-17-acetoxy-4,4,10,13-tetramethyl-7-oxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta (a) phenanthren-3-yl was found to show better docking interaction than the commercial standard drugs. This study also showed that molecular dynamics simulation can be used as a tool to predict the binding mode and the affinity of compounds to the target proteins in a relatively accurate manner. However, further in vitro and in vivo studies are required to validate the potential of sea anemone derived compounds. Screening novel enoyl-acyl



(a) Acetic acid-17-acetoxy-4,4,10,13-tetramethyl-7-oxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta (a) phenanthren-3-yl (ester).



(b) 4[-4-Diethylamino-1-methylbutylamino]-1, 2 dimethoxy-6-bromonaphthalene.



(c) Tryptanthrine

Fig. 8 2D molecular interaction between the target receptor and the ligand molecule along with the neighboring residues.

Fig. 9 Pymol molecular visualization of the ligand and the target protein

carrier protein reductase pathway inhibitors could be done by molecular docking based virtual screening followed by molecular dynamics simulations.

Acknowledgements Authors are thankful to Dean & Director, Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences and authority of Annamalai University for providing necessary facilities. First author sincerely acknowledged the DBT and UGC-BSR for their financial support.

Funding Funding was provided by DBT and UGC-BSR projects.

Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interest.

Ethical Approval The Institutional Ethical Committee of Rajah Muthiah Medical College, Annamalai University, Annamalai Nagar, India (registration number 160/1999/CPCSEA/11.01.2008) approved and provided the ethical clearance for the present study.

References

Ali MA, Shaharyar M (2007) Discovery of novel phenoxyacetic acid derivatives as anti-mycobacterial agents. Bioorg Med Chem 15:1896–1902

Alvarez C, Mancheno JM, Martinez D, Tejuca M, Pazos F, Lanio ME (2009) Sticholysins, two pore-forming toxins produced by the Caribbean Sea anemone *Stichodactyla helianthus*: their interaction with membranes. Toxicon 54(8):1135–1147

Anderluh G, Macek P (2002) Cytolytic peptide and protein toxins from sea anemones *Anthozoa: Actiniaria*. Toxicon 40:111–124

Bala SRG, Venkata RD, Rao CHB, Dhananjaya N, Kuttan R, Babu TD (1999) Isolation and structural determination of new sphingolipids and pharmacological activity of Africanene and other metabolites from *Sinularia leptoclados*. Chem Pharm Bull 47:1214–1220

Balamurugan E, Menon VP (2009) In vitro radical scavanging activities of *Chrysaora quinquecirrha* nematocyst venom. Drug Discov Ther 3(2):56–61

Bates I, Fenton C, Gruber J, Lalloo D, Lara AM, Squire SB, Theobald S, Thomson R, Tolhurst R (2004) Lancet Infect Dis 4:368–375

Battison AL, Summerfield R, Patrzykat A (2008) Isolation and characterisation of two antimicrobial peptides from hemocytes of the American lobster *Homarus americanus*. Fish Shellfish Immunol 25:181–187

Beress L, Beress R (1971) Reinigung zweier krabbenlahmender toxins asunder sea anemone *Anemonia sulcata*. Kiel Meeresforsch 27:117–127

Bragadeeswaran S, Thangaraj S, Rajak RC, Balaji D (2011) Pharmacological and biomedical properties of sea anemones *Paracondactylis indicus, Paracondactylis sinensis, Heteractis magnifica* and *Stichodactyla haddoni* from east coast of India. Asian Pac J Trop Med 4:722–726

Brooijmans N, Kuntz ID (2003) Molecular recognition and docking algorithms. Annu Rev Biophys Biomol Struct 32:335–373

Brumfitt W, Hamilton JMT, Franklin I (1990) Antibiotic activity of natural products: 1. Propolis. Microbios 62:19–22

Buckley LJ, Ikawa M, Sasner JJJ (1975) Purification of two *Gonyaulax tamarensis* toxins from clams *Mya arenaria* and the identification of STX. Toxic dinoflagellate blooms. Proc Int Conf Mass Sci Technol Found 1975:423–431

Bursulaya B, Totrov M, Abagyan R, Brooks C (2003) J Comput Aided Mol Des 17:755–763

Chen WT, Li Y, Guo YW (2012) Terpenoids of *Sinularia* soft corals: chemistry and bioactivity. Acta Pharm Sinica B 2(3):227–237

Devi KN, Kumar TTA, Dhayanithi NB, Kathiresan K (2012) Isolation of pigments from sea anemones, *Heteractis magnifica* and *Stichodactyla haddoni* and their effects against aquatic and human bacterial pathogens. Asian Pac J Trop Biomed 2:S323–S328

Duan XJ, Zhang WW, Li XM, Wang BG (2006) Evaluation of antioxidant property of extract and fractions obtained from a red alga *Polysiphonia urceolata*. Food Chem 95:37–43

Encarnacion DR, Franszblau SG, Tapia CA, Cedillo-Rivera R (2000) Screening of marine organisms for antimicrobial and antiprotozoal activity. Pharm Biol 38:379–384

Ewing T, Makino S, Skillman A, Kuntz I (2001) DOCK 4.0: search strategies for automated molecular docking of flexible molecule databases. J Comput Aided Mol Des 15(5):411–428

Faulkner DJ (2001) Marine natural products. Nat Prod Rep 18:1–49

Ferlan I, Lebez D (1974) Equinatoxin, a lethal protein from *Actinia equina*-I. Purification and characterization. Toxicon 12:57–61

Ferlan I, Levez D (1976) Preliminary studies on the structure of equinatoxin. Bull Inst Pasteur 74:121

Freundlich JS, Wang F, Vilcheze C, Gulten G, Langley R, Schiehser GA, Jacobus DP, Jacobs WRJ, Sacchettini JC (2009) Triclosan derivatives: towards potent inhibitors of drug-sensitive and drug-resistant *Mycobacterium tuberculosis*. Chem Med Chem 4(2):241–248

Galeano E, Martinez A (2007) Antimicrobial activity of marine sponges from Uraba Gulf, Colombian Caribbean region. J Mycol Med 17:21–24

Geffen Y, Rosenberg E (2005) Stress-induced rapid release of antibacterial by scleractinian corals. Mar Biol 146:931–935

Griffin SP, Bhagooli R (2004) Measuring antioxidant potential in corals using the FRAP assay. J Exp Mar Biol Ecol 302:201–211

Gulcin I, Beydemir S, Sat G, Kufrevioglu OI (2005) Evaluation of antioxidant activity of cornelian cherry (*Cornus mas* L.). Acta Aliment 34(2):193–202

Harder T, Lau SCK, Dobretsov S, Fang TK, Qian PY (2003) A distinctive epibiotic bacterial community on the soft coral *Dendronephthya* sp. and antibacterial activity of coral tissue extracts suggest a chemical mechanism against bacterial epibiosis. FEMS Microbiol Ecol 43:337–347

Hawa I, Zulaikah M, Jamaludin M, Zainal Abidin AA, Kaswandi MA, Ridzwan BH (1999) The potential of the coelomic fluid in sea cucumber as an antioxidant. Malays J Nutr 5:55–59

Honma T, Shiomi K (2005) Peptide toxins in sea anemones: structural and functional aspects. Mar Biotechnol 8:1–10

Jensen PR, Harvell CD, Wirtz K, Fenical W (1996) Antimicrobial activity of extracts of Caribbean gorgonian corals. Mar Biol 125:411–419

Jinhua F, Huahua Y, Cuiping L, Ronge X, Song L, Lin W, Shengbao C, Pengcheng L (2009) Isolation and characterization of venom from nematocysts of jellyfish *Rhopilema esculentum* Kishinouye. Chin J Oceanol Limnol 27:869–874

Jorgensen WL (2004) The many roles of computation in drug discovery. Science 303:1813–1818

Kapoor M, Reddy CC, Krishnasastry MV, Surolia N, Surolia A (2004) Slow-tight-binding inhibition of enoyl-acyl carrier protein reductase from *Plasmodium falciparum* by triclosan. Biochem J 381:719–724

Kem WR, Parten B, Pennington MW, Price DA, Dunn BM (1989) Isolation, characterization, and amino acid sequence of a polypeptide neurotoxin occurring in the sea anemone *Stichodactyla helianthus*. Biochemistry 28:3483–3489

Kitchen DB, Decornez H, Furr JR, Bajorath J (2004) Nature reviews. Drug Discov 3(11):935–949

Koh LL, Tan TK, Chou LM, Goh NKC (2002) Antifungal properties of Singapore gorgonians: a preliminary study. J Exp Mar Biol Ecol 273:121–130

Kohno Y, Satoh H, Iguchi A, Nagai H (2009) Characterization of a new hemolytic protein toxin from the sea anemone *Anthopleura asiatica*. Fish Sci 75:1049–1054

Kristan K, Viero G, Dalla Serra M, Macek P, Anderluh G (2009) Molecular mechanism of pore formation by actinoporins. Toxicon 15(8):1125–1134 54)

Kuntz ID, Meng EC, Shoichet BK (1994) Structure-based molecular design. Acc Chem Res 27(5):117–123

Lin T-W, Melgar MM, Kurth D, Swamidass SJ, Purdon J, Tseng T, Gago G, Baldi P, Gramajo H, Tsai SC (2006) Structure-based inhibitor design of AccD5, an essential acyl-CoA carboxylase carboxyltransferase domain of *Mycobacterium tuberculosis*. Proc Natl Acad Sci 103(9):3072–3077

Ling LL, Xian J, Ali S, Geng B, Fan J, Mills DM, Arvanites AC, Orgueira H, Ashwell MA, Carmel G, Xiang Y, Moir DT (2004) Identification and characterization of inhibitors of bacterial enoyl-acyl carrier protein reductase. Antimicrob Agents Chemother 48(5):1541–1547

Mamelona J, Pelletier E, Lalancette KG, Legault J, Karboune S, Kermasha S (2007) Quantification of phenolic contents and antioxidant capacity of Atlantic sea cucumber *Cucumaria frondosa*. Food Chem 104(3):1040–1047

Marquis CP, Baird AH, de Nys R, Holmstrom C, Koziumi N (2005) An evaluation of the antimicrobial properties of the eggs of 11 species of scleractinian corals. Coral Reefs 24:248–253

Ospina CA, Rodriguez AD (2006) Bioactive compounds from the gorgonian *Briareum polyanthes*. Correction of the structures of four asbestinane-type diterpenes. J Nat Prod 69(12):1721–1727

Oyaizu M (1986) Studies on products of browning reaction prepared from glucoseamine. Jpn J Nutr 44:307–314

Patton WK (1995) Three dimensional structure in solution of neurotoxin III from the sea anemone Anemonia sulcata. Sea anemones. Academic American Encyclopedia, ed. Manoleras, N, 4. R. S. Norton

Prieto P, Pineda M, Aguilar M (1999) Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal Biochem 269:337–341

Rock CO, Cronan JE (1996) *Escherichia coli* as a model for the regulation of dissociable (type II) fatty acid biosynthesis. Biochim Biophys Acta 1302:1–16

Rojas A, Torres M, Rojas J, Peregrino A, Heimer-de la Cotera EP (2002) Calcium-dependent smooth muscle excitatory effect elicited by the venom of the hydrocoral *Millepora complanata*. Toxicon 40:777–785

Rottini G, Gusmani L, Parovel E, Avian M, Patriarca P (1995) Purification and properties of a cytolytic toxin in venom of the jellyfish *Carybdea marsupialis*. Toxicon 33(3):315–326

Roussis V, Chinou IB, Tsitsimpikou C, Vagias C, Petrakis PV (2001) Antibacterial activity of volatile secondary metabolites from Caribbean soft corals of the genus Gorgonia. Flavour Frag J 16:364–366

Rozwarski DA, Grant GA, Barton DH, Jacobs WRJ, Sacchettini JC (1998) Modification of the NADH of the isoniazid target (InhA) from *Mycobacterium tuberculosis*. Science 279(5347):98–102

Ryan KJ, Ray CG (2004) Sherris medical microbiology. McGraw Hill, New York

Sahu SK, Kathiresan K, Singh R, Senthilraja P (2012) Molecular docking analyses of *Avicennia marinaderived* phytochemicals against white spot syndrome virus (WSSV) envelope protein-VP28. Bioinformation 8(18):897–900

Santamaria A, Sanchez-Rodriguez J, Zugasti A, Martinez A, Galvan-Arzate S, Segura-Puertas L (2002) A venom extract from the sea anemone *Bartholomea annulata* produces haemolysis and lipid peroxidation in mouse erythrocytes. Toxicol 173:221–228

Shoichet BK, Kuntz ID, Bodian DL (2004) J Comp Chem 13(3):380–397

Singleton VL, Orthofer R, Lamuela-Raventos RM (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol 299:152–178

Smith S, Witkowski A, Joshi AK (2003) Structural and functional organization of the animal fatty acid synthase. Prog Lipid Res 42:289–317

Suganthi K, Bragadeeswaran S, Sri Kumaran N, Thangaraj S, Balasubramanian T (2011) Biological and pharmacological activities of jelly fish *Crambionella stuhalmanni* and *Chrysaora quinquecirrha*. Inter. J Pharm Pharm Sci 3(2):230–236

Tadesse M, Gulliksen B, Strom MB, Styrvold OB, Haug T (2008) Screening for antibacterial and antifungal activities in marine benthic invertebrates from northern Norway. J Invertebr Pathol 99:286–293

Takayama K, Wang C, Besra GS (2005) Pathway to synthesis and processing of mycolic acids in Mycobacterium tuberculosis. Clin Microbiol Rev 18:81–101

Thangaraj S, Bragadeeswaran S (2012) Assessment of biomedical and pharmacological activities of sea anemones *Stichodactyla mertensii* and *Stichodactyla gigantea* from Gulf of Mannar Biosphere Reserve, southeast coast of India. J Venom Anim Toxins incl Trop Dis 18(1):53–61

Thangaraj S, Bragadeeswaran S, Suganthi K, Sri Kumaran N (2011) Antimicrobial properties of sea anemone *Stichodactyla mertensii* and *Stichodactyla gigantea* from Mandapam coast of India. Asian Pac J Trop Biomed 1:S43–S46

Torres M, Aguilar MB, Falcon A, Sanchez L, Radwan FF, Burnett JW, Heimer-de la Cotera EP, Arellano RO (2001) Electrophysiological and hemolytic activity elicited by the venom of the jellyfish *Cassiopea xamachana*. Toxicon 39(9):1297–1307

Vilcheze C, Wang F, Arai M, Hazbon MH, Colangeli R, Kremer L, Weisbrod TR, Alland D, Sacchettini JC, Jacobs WRJ (2006) Transfer of a point mutation in *Mycobacterium tuberculosis* InhA resolves the target of isoniazid. Nat Med 12(9):1027–1029

Wei LS, Musa N, Wee W, Musa N, Seng CT (2007) Antimicrobial property of 12 spices and methanolic extract of ornamental sea anemone *Radianthus ritteri* against Edwardsiellosis agent and other bacteria. Adv Biol Res 1(5–6):164–166

Williams GP, Babu S, Ravikumar S, Kathiresan K, Arul Prathap S, Chinnapparaj S, Marian MP, Alikhan SL (2007) Antimicrobial activity of tissue and associated bacteria from benthic sea anemone *Stichodactyla haddoni* against microbial pathogens. J Environ Biol 28(4):789–793

Wilsanand V, Wagh AB, Bapuji M (1999) Antibacterial activities of anthozoan corals on some marine microfoulers. Microbios 99(394):137–145

World Health Organization (2010) Global tuberculosis control 2010, WHO report 2010. World Health Organization, Geneva, p. 5

Yu H, Liu X, Xing R, Liu S, Guo Z, Wang P, Li C, Li P (2006) In vitro determination of antioxidant activity of proteins from jellyfish *Rhopilema esculentum*. Food Chem 95:123–130

Yu H, Li C, Li R, Xing R, Liu S, Li P (2007) Factors influencing hemolytic activity of venom from the jellyfish *Rhopilema esculentum* Kishinouye. Food Chem Toxicol 45:1173–1178

Zhang H, Yang Z, Shen Y, Tong L (2003) Crystal structure of the carboxyltransferase domain of acetyl-coenzyme A carboxylase. Science 299:2064–2067