Journal of Pharmacy and Alternative Medicine
ISSN 2222-5668 (Paper) ISSN 2222-4807 (Online)
Vol. 2, No. 3, 2013

Research Article

Bioactive potential of sea urchin *Temnopleurus toreumaticus* from Devanampattinam, Southeast coast of India

S. Bragadeeswaran[1]*, N. Sri Kumaran[1], P. Prasath Sankar[1], R. Prabahar[1]

[1]Centre of Advanced Study in Marine Biology, Faculty of Marine Science, Annamalai University, Parangipettai - 608 502, Tamil Nadu, India

*E-mail of the corresponding author: drpragathi@gmail.com

Accepted Date: 27 August 2013

Abstract

The present investigation elucidates the bioactive potential of aqueous extract of sea urchin *Temnopleurus toreumaticus*. In this investigation biochemical, heamolytic, cytotoxic and FT-IR analysis were followed by standard methods. In biochemical analysis, proteins content 2.70 mgmL^{-1}, total carbohydrates content 2.15 mgmL^{-1} and total lipids content 0.03 mgmL^{-1} were showed in aqueous extract of sea urchin. In heamolytic assay, the goat and chicken erythrocytes showed the maximum 64 Hemolytic Unit (HU) and human blood group "A" shows 32 HU, blood group "B" shows 64 HU, blood group "AB" shows 32 HU and blood group "O" shows 128 HU. In cytotoxic study, aqueous extract of sea urchin showed LC_{50} value 0.12±0.09 mgmL^{-1} concentration was showed 50% mortality. In antimicrobial assay, maximum zone inhibition 12.26 ± 0.6 mm showed by *K. oxytoca* and 3.33 ± 0.9 mm showed by *Mucor* sp. against chloroform extract of sea urchin *T. toreumaticus*. Thin layer chromatogram showed the spots of *Rf* values of 0.38, 0.85 cm. The FT-IR study shows the presence of functional groups such as chloroalkanes, bromoalkanes, iodoalkanes, alcohols groups, acids or aromatic ethers, methyl alkyl groups, 1° amines groups and ammonium ions. These results indicate that, sea urchin has remarkable hemolytic and cytotoxic activities.

Keywords: Antimicrobial Assay, Biochemical, FT-IR, Sea Urchin, Heamolytic

1. Introduction

The marine environment is an exceptional reservoir of bioactive natural products, many of which exhibit structural and chemical features not found in terrestrial natural products. The richness of diversity offers a great opportunity for the discovery of new bioactive compounds. Modern technologies have opened vast areas of research for the extraction of biomedical compounds from ocean and seas to treat the deadly diseases. The number of natural products isolated from marine organisms increases rapidly, and now exceeds with hundreds of new compounds being discovered every year (Proksch and Muller, 2006).

The secondary metabolites have various functions; it is likely that some of them may be pharmacologically active on humans and useful as medicines (Briskin, 2000). A majority of pharmacologically active secondary metabolites have been isolated from echinoderms (Carballeria et al., 1996). There are much valuable information for new antibiotic discoveries and give new insights into bioactive compounds in sea urchin. The sea urchin shells are containing various polyhydroxylated naphtoquinone pigments, spinochromes (Anderson et al., 1969) as well as their analogous compound, echinochrome A, of which was shows bactericidal effect was reported by Service et al. (1984). The phenolic hydroxyl groups in these molecules also suggested that they could participate in antioxidant activity as was observed in other well-known antioxidant poly phenols such as tea catechins. The similar structured compounds are also found in the shells of sea urchins and thus suggesting that they as well as echinochrome A would act as antioxidant substances similar to other olyphenolic antioxidants in edible plants (Chantaro et al., 2008). While squaric acid ester-based methodology was used in a new synthesis of echinochrome A, a polyhydroxylated napthoquinone pigment commonly isolated from sea urchin spines (Pena-Cabrera et al., 2002). The Sea urchin gonads polyhydoxylated naphthoquinone, echinochrome A, of which potential in

antioxidant activity (Lebedev et al., 2001).

The present investigation was undertaken to study the bioactive potential in crude extract of sea urchin (*T. toreumaticus).* The potential of echinoderms as a source of biologically active products are largely unexplored. Hence, a broad, based screening of sea urchin for bioactive compounds is necessary. Many marine invertebrates are potentially vulnerable to microbial infection. The literature survey revealed the little is known about the biomedical activities from sea urchins. Hence, the present study was undertaken to investigate biomedical potential of sea urchin *T. toreumaticus* from Devanampattinam, Southeast coast of India.

2. Materials and Methods

2.1. Specimen collection

The sea urchin *T. toreumaticus* was collected from Devanampattinam (Lat 11º44'N; Long 79º47'E) landing centre from Cuddalore district, Tamil Nadu, Southeast coast of India. The samples were thoroughly washed with sea water to remove sand, mutt and overgrowing organisms at the collection site and transported to laboratory. The collected specimens were identified by the standard literature of taxonomic guide by Clark and Rowe, (1971). The collected specimens were immediately shade dried.

2.2. Crude extract preparation

The sea urchin *T. toreumaticus* (195 g) was soaked in distilled water (250 mL) and maintained for 12 h. The extract was filtered through Whatman®No.1. Filter paper and lyophilized and stored at - 4℃ for further analysis.

2.3. Protein estimation

Protein estimation followed by the method of Bradford, (1976). Bovine serum albumin (2 $mgmL^{-1}$) was used as reference standard at various concentrations viz., 20, 40, 60, 80 and 100 µg/100 µL. The assay relies on the binding of the dye Coomassie Blue G250 to the protein molecule measured calorimetrically at 595 nm.

2.4. Carbohydrate estimation

The total carbohydrate was estimated by following Phenol-sulphuric acid method of Dubois et al., (1956). Glucose (1 μgmL^{-1}) was used as a as reference standard concentrations 20, 40, 60, 80 and 100 µg/100 µL. The absorbance was measured at 490 nm in a spectrophotometer (HITACHI-220S UV).

2.5. Lipids estimation

The lipid estimation was determined by the method of Folch et al., (1957).

2.6. Hemolytic assay

The aqueous extract of the sea urchin *T. toreumaticus* was tested on chicken, goat and human blood group (A, B, AB and O) erythrocytes according to the method of Bragadeeswaran et al., (2011). This assay was performed on a 'V' shaped sterile Laxbro microtitre plate (India). Serial two-fold dilutions of the venom 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% RBC was added to each well. The plate was shaken to mix the RBC with aqueous extract. The plates were incubated at room temperature for two hours before reading the results. Appropriate control was included in the tests. Erythrocyte suspensions to which 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.

2.7. Cytotoxicity activity using brine shrimp larvae

The cytotoxicity assay was performed using the method of Meyer et al., (1982) with *A. salina* (24 hrs post hatching). Brine shrimp eggs were hatched in a beaker filled with filtered sea water under constant aerator. After 48 hrs, the phototropic nauplii were collected by pipette. The nauplii were counted macroscopically in the stem of pipette against a lighted background. 10 shrimps were transferred to each concentration of aqueous extract, which were 0.025, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 $mgmL^{-1}$ subjected to 24 hrs

exposures under illuminations. Survivors were counted after 24 hrs of incubation and the percentage of deaths at each concentration were counted and control (sea water) was maintained with test organisms.

2.8. Antimicrobial activity

2.8.1. Extract preparation for microbial activity

The lyophilized crude extract 1grm of sea urchin *T. toreumaticus* was dissolved in each 1 mL in PBS, methanol, n-butanol, chloroform and petroleum ether solvents.

2.8.2. Microorganisms

The bacterial pathogens such as *E. coli*, *K. oxytoca*, *K. pneumoniae*, *K. aeruginosa*, *P. mirabilis*, *S. paratyphi*, *S. typhi*, *Staphylococcus aureus*, *V. cholerae* and *V. parahaemolyticus*, the fungal pathogens such as *Alternaria* sp., *A. flavus*, *A. niger*, *C. albicans*, *C. tropicalis*, *Mucor* sp., *Penicillium* sp., *Rhizopus* sp., T. *mentagarophytes* and *T. rubrum* were used for antimicrobial assay. These pathogens were obtained from Rajah Muthiah Medical College and Hospital, Annamalai University, Annamalainagar. The bacterial and fungal strains were maintained on nutrient agar and fungal agar slants at 4 ℃ respectively.

2.8.3. Antibacterial activity

Antibacterial activity was carried out by using standard disc diffusion method by Sri Kumaran et al., (2011). The test cultures (bacteria 108 $CFUmL^{-1}$) were swabbed on top of the solidified media and allowed to dry for 10 mins. The human bacteria were maintained on nutrient agar plates. The different extracts were applied on to 6 mm sterile discs in aliquots of 30 µL of solvent, allowed to dry at room temperature and extract loaded discs were placed on agar plates seeded with isolated microorganisms and incubated at 37 ℃ for 24 hrs. The susceptibility of the test organisms were determined by radius of the zones inhibition around each disc. The tetracycline discs were used as a positive control and solvents discs were used as a negative control.

2.8.4. Antifungal activity

Antifungal activity was carried out by using the standard disc diffusion method by National Committee for Clinical Laboratory Standards, (2006).

2.9. Thin layer chromatography (TLC)

The aqueous sample were analysed by TLC coupled to chemical tests for identification of different secondary metabolites according to MINSAP, (1995). For analytical TLC, aluminium sheets (4×5 cm) coated with silica gel 60 F254, were used. The chromatography was run in a chamber with methanol: chloroform: water as a medium (6:4:4 v/v) as a mobile phase under UV light at 254 nm.

2.10. Fourier transform infrared spectroscopy (FT-IR)

The powdered samples (5 mg) were mixed with dry KBr (100 mg) and subjected to a pressure of about 5.106 Pa in an evacuated die to produce a clear transparent disc of 13 mm diameter and 1 mm thickness. FT-IR spectra in the region 4000-500 cm^{-1} were recorded at room temperature (25± 1℃) on a Nicolet-Avater-360. FT-IR spectrometer equipped with an air-cooled DTGS (Deuterated Triglycine Sulfate) and purged with nitrogen. For each spectrum 100 interferograms providing a spectral resolution of 4 cm^{-1} were co-added. Each sample was scanned with three different pellets under identical conditions. The spectra were analyzed using ORIGIN 8.0 software.

3. Results

The collected urchin *T. toreumaticus* were identified the standard method of the taxonomic guide by Clark and Rowe, (1971). In the present investigation aqueous extract of *T. toreumaticus* was lyophilized to give an 11.67 g white powder. In biochemical analysis, proteins content 2.70 $mgmL^{-1}$, total carbohydrates content 2.15 $mgmL^{-1}$ and total lipids content 0.03 $mgmL^{-1}$ were showed in aqueous extract of sea urchin *T. toreumaticus*. In heamolytic assay the goat and chicken blood showed the maximum of 64 Hemolytic Unit (HU) (Fig. 1). Figure. 2. shows the heamolytic effects on human erythrocytes. In this assay blood group A shows 32 HU, blood group B shows 64 HU, blood group AB shows 32 HU and blood group O shows 128 HU (Fig. 2).

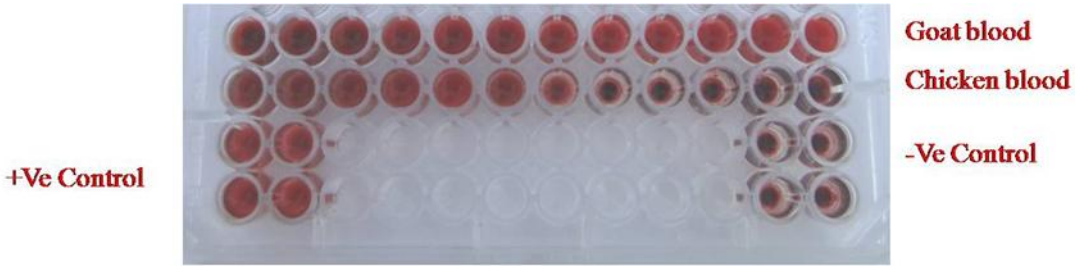


Figure 1: Hemolytic effects from the aqueous crude extract of sea urchin *T. toreumaticus* on chicken and goat erythrocytes.

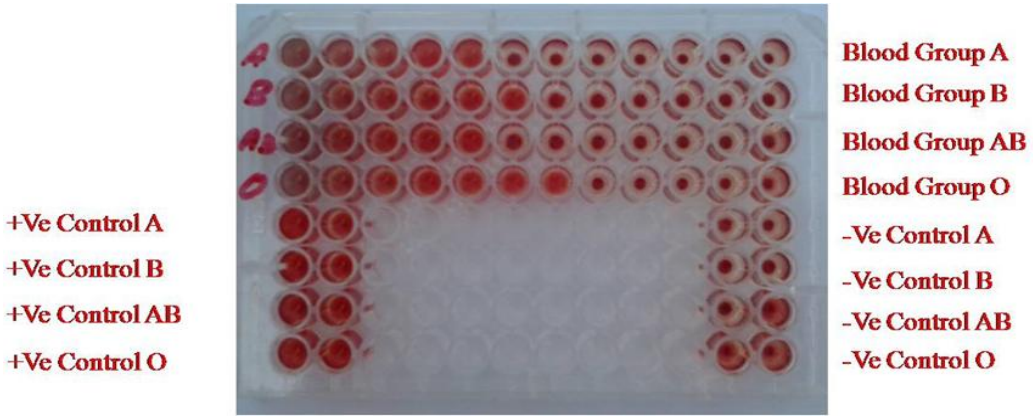


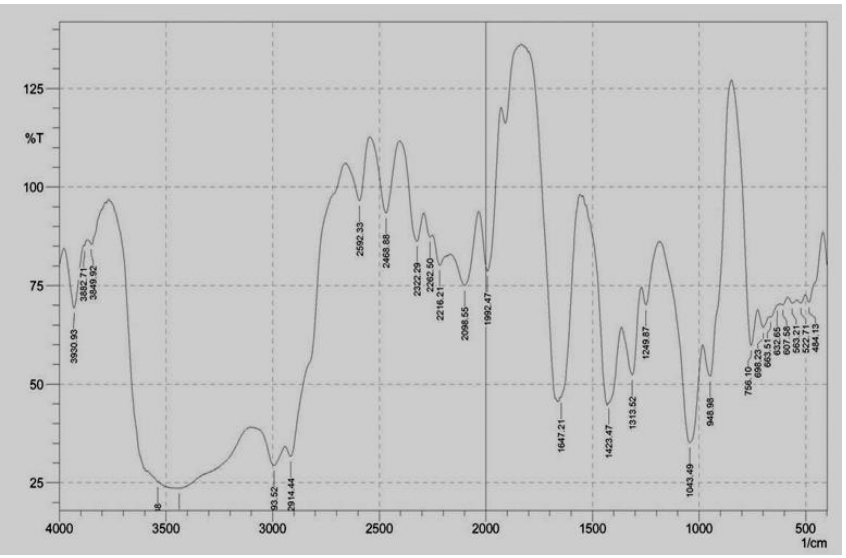
Figure 2: Hemolytic effects from the aqueous crude extract of sea urchin *T. toreumaticus* on human erythrocytes.

The aqueous extract of sea urchin *T. toreumaticus* was tested cytotoxic activity against *A. salina* larvae. The brine shrimp assay is considered as a consistent indicator for the preliminary evaluation of toxicity. The LC_{50} values are given in (Table 1). In this assay the LC_{50} value was observed between the 0.1 to 0.2 $mgmL^{-1}$, that means the 0.12 ± 0.09 $mgmL^{-1}$ concentration from crude extract of *T. toreumaticus* was express the 50% mortality of the *A. salina*.

The antibacterial activity and the inhibition growth of bacteria and fungi from the extract of sea urchin *T. toreumaticus* were given in Table. 2. In the present study, sea urchin *T. toreumaticus* extracts showed moderated activity against tested pathogens. In this assay the bacterial pathogen *K. oxytoca* shows high inhibition zones (12.26 ± 0.6 mm) against chloroform extract. In case of fungal pathogens *Mucor* sp. shows highest sustainability (3.33 ± 0.9 mm) against chloroform extract (Table 2).

The aqueous extract was loaded on silica gel glass plates for the purification and characterization of active compounds. Thin layer chromatogram showed the presence of spots with *Rf* values of 0.38, 0.85 cm in aqueous extract (Fig. 3). The FT-IR study was carried for identify the functional groups of the aqueous extract of sea urchin *T. toreumaticus* (Fig. 4). In FT-IR study 484-756 cm^{-1} represent the presence of chloroalkanes, bromoalkanes, iodoalkanes, 948 cm^{-1} represent of monosubstituted alkenes, between 1040–1060 cm^{-1} shows the presence of alcohols groups (strong, broad). The 1249 cm^{-1} shows the presence of carboxylic acids or aromatic ethers. The 1313 cm^{-1} shows the methyl alkyl groups in it. 1423 cm^{-1} shows the presence of aromatic C═C, 1647 cm^{-1} shows the presene of amine group 1 (N–H) bend 1° amines groups. 2098 cm^{-1} (w) shows the presence of –C ≡ C– stretch alkynes groups. The range between 2260 - 2100 shows the presence of –C ≡ C– stretch alkynes groups. The 2468 $cm^{-1}$and 2592 cm^{-1} shows the presence of ammonium ions in this compound. The 2914 cm^{-1}, 2993 cm^{-1} shows the presence of C–H

stretch alkanes in this sea urchin *T. toreumaticus* extract.



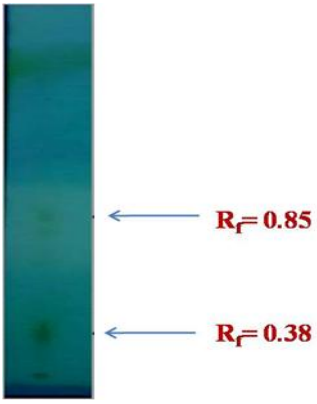


Figure 3 (Right): Showing the TLC of aqueous sea urchin *T. toreumaticus* extract (*Rf* values =0.38, 0.85) on Methanol: Chloroform Water (6:4:4 v/v) solvent system under UV light.

Figure 4 (Left): FT-IR analysis from aqueous extract of sea urchin *T. toreumaticus*.

Table 1: Cytotoxicity effect from aqueous extract of sea urchin *T. toreumaticus*

Concentration (mgmL^{-1})	**aqueous extract of sea urchin *T. toreumaticus***
Control	0
0.025	0
0.05	2.33±0.58
0.1	3.66±1.15
0.2	6.33±0.58
0.4	9
0.6	9.66±0.58
0.8	10
1.0	10
LC_{50}	**0.12±0.09**

Table 2: Antimicrobial activity of sea urchin *T. toreumaticus* against human pathogens

Human Bacterial Pathogen	Anti microbial activity (mm)					
	PBS	Methanol	Chloroform	n-Butanol	Petroleum ether	+ve Control
E. coli	2.01±0.01	2.05±0.1	4.02±0.1	1±0.11	-	20.01±0.6
K. oxytoca	3.2±0.1	2.06±0.11	12.26±0.6	3.04±0.25	-	19.04±0.4
K. pneumoniae	-	1.04±0.15	5.11±0.15	4.02±0.6	-	22.08±0.11
P. aeruginosa	-	-	5.01±0.1	2.05±0.14	-	22.15±0.5
P. mirabilis	-	-	11.07±0.4	3.01±0.4	-	18.12±0.6
S. paratyphi	-	3±0.15	10.05±0.3	2.06±0.3	-	17.1±0.19
S. typhi	-	-	10.04±0.12	2.3±0.14	-	18.33±0.17
S. aureus	-	-	3.06±0.11	2.09±0.18	-	15.17±0.5
V. cholerae	-	-	7.01±0.14	4.12±0.14	-	11.19±0.01
V. parahaemolyticus	-	1.01±0.1	8.05±0.4	2.18±0.1	-	22.11±0.4
Fungal pathogens						
Alternaria sp.	-	-	3.04±0.6	-	-	17.16±0.14
A. flavus	-	-	3.05±0.4	-	-	11.17±0.2
A. niger	1±0.5	-	2.21±0.5	-	-	10.11±0.01
C. albicans	-	-	2.15±0.4	-	-	11.14±0.3
C. tropicalis	-	-	1.04±0.22	-	-	14.08±0.14
Mucor sp.	-	-	3.33±0.9	-	-	12.19±0.41
Penicillium sp.	-	-	1.05±0.4	-	-	10.1±0.33
Rhizopus sp.	1.02±0.6	-	3.03±0.18	-	-	22.2±0.12
T. mentagarophytes	-	-	2.04±0.11	-	-	9.45±0.14
T. rubrum	2.06±0.4	-	3.05±0.4	-	-	13.11±0.6

4. Discussion

In recent years, great attention has been paid to study the bio-activity of natural products due to their potential pharmacological utilization. Echinoderms with special regard to sea urchins generally contain one or more cytolytic peptides or proteins. The sea urchin, 100 g worth or 3.5 oz, have 128 g calories, 12 g of protein and almost 3 g of fat. As many sea foods, the sea urchin is low in calories and has a good amount of Omega 3 fatty acids. The edible parts of the sea urchin are the 5 sex glands or tongues and the coral and liquid surrounding those glands. In the present study biochemical analysis such as protein contents 2.70 $mgmL^{-1}$, carbohydrate contents 2.15 $mgmL^{-1}$ and lipid contents 0.03 $mgmL^{-1}$ were observed from aqueous extract of *T. toreumaticus*.

The rationale of searching for drugs from marine environment stems from the fact that marine plant and animals have adapted to all sorts of marine environment and these creatures are constantly under tremendous selection pressure including space competition, predation, surface fouling and reproduction. Many of these organisms have been antimicrobial properties, although most of the anti-bacterial agents that

have been isolated from marine sources have not been active enough to complete with classical antimicrobial obtained from micro organisms (Rinehart et al., 1981). However, majority of marine organisms are yet to be screened for discovering useful antibiotics. The results suggest that the sea urchin can produce antimicrobial and cytotoxic substances instantly tons combat microbial infection. In the present study the sea urchin extract shows promising result against tested human bacterial and fungal pathogens. Several drug discovery projects have screened echinoderms for antibiotic activities. The adult echinoderms have a few major organs such as mouth parts, intestine, nerve ring, and gonad. These organs are located in the cavity, named celomic cavity. The remaining space of the celomic cavity is filled with celomic fluid which contains various "blood cells", the celomocytes. These cells are though to mediate the main immune functions in echinoderms, including antimicrobial activities. Many studies have been conducted to examine the activity of celomocytes, lysates and celomic fluid against bacteria, fungi and even tumour cells (Li et al., 2010). Antibacterial activity was detected in extracts of several tissues from the green sea urchin *S. droebachiensis*, the common sea star Asterias rubens, and the sea cucumber *Cucumaria frondosa* (Haug et al., 2002). It is an interesting finding that sea urchin being marine animal has the ability to dispose the bacteria upon infection. As the bacterium is a human pathogen, it is important that sea water should not be in the water and the peptides can kill more efficient than the conventional antibiotics.

The lyophilized aqueous extract *of T. toreumaticus* was showed promising hemolytic activities in chicken and goat and various human blood erythrocytes. The activity was slightly differed considerably the type of erythrocytes. This may be due to the presence of hemolysin present in the red blood cells and permeability of cell membranes. Many biotoxins are known to cause heamolysis of RBC and chemical poison may also cause excessive hemolysis. Hemolytic activity has been observed in many of the tissue products of aquatic organisms. Catfish skin toxin and the venom from their dorsal and pectoral spines have been reported to be hemolytic activity (Mann and Werntz, 1991). Sri Kumaran et al., (2012) observed n-butanol extract of ascidian *L. fragile* showed high hemolytic activity 64 HU against chicken erythrocytes, 16 HU in goat erythrocytes and 16 HU in cow erythrocytes. Bragadeeswaran et al., (2011) observed the hemolytic activity 32 HU, 16 HU of ascidian *P. madrasensis* and *P. nigra* extracts, against chicken erythrocytes, 8 HU and 16 HU, in human blood group A erythrocytes, 2 HU and 32 HU in human blood group B erythrocytes, 32 HU in human blood group AB erythrocytes, 2 HU and 32 HU in human blood group O erythrocytes receptively. Thangaraj and Bragadeeswaran, (2012) observed maximum heamolytic activity 32 HU in sea anemones extract *S. mertensii* and *S. gigantea* against chicken erythrocytes. The observation made by the present findings has coincided with the above investigations.

Some compounds exhibited hemolytic activity as well as toxicity towards to brine shrimps. In this cytotoxic study, the sea urchin *T. toreumaticus* aqueous extract were tested against brain shrimps; in that aqueous extract showed LC_{50} value at 0.12 ± 0.09 $mgmL^{-1}$ concentration showed 50% mortality. Cytotoxic activity has been observed in many of the tissue products of aquatic organisms. There were several reports on cytotoxic activities observed in marine algae and ascidians. Mtolera and Semesi, (1996) tested cytotoxic activity six marine algae, from that *U. pertusa* extract showed high LC_{50} value 116 μgmL^{-1}, whereas extracts of *Caulerpa racemosa* and *Valonia aegrophila* showed LC_{50} above 1000 μgmL^{-1}. The epidioysterol is toxic against *A. salina* larva, derived from morocco ascidian, *C. savignyi*; LC_{50} value showed 71 μgmL^{-1}. Sri Kumaran et al. (2012) observed n-butanol extract of *L. fragile* showed high LC50 97 μgmL^{-1}. The present observation has coincided with above described studies. Previously, the antibacterial activity has been described in a broad range of echinoderm species (Ridzwan et al., 1995). In most of the species studied, the whole body or body wall has been tested for activity.

In the present study maximum zone inhibition (12.26 ± 0.6 mm) was showed by *K. oxytoca* and high sensitivity (3.33 ± 0.9 mm) was showed by *Mucor* sp. against chloroform extract of sea urchin *T. toreumaticus*. This similar study conducted by Uma and Parvathavarthini, (2010) from the hexane extract of sea urchin *T. alexandri*, in this study the zone of inhibition was found to increase with increased concentration of the extract. Of all the concentrations tested, 5000 ppm was showed to have greater antibacterial activity than the other concentrations (5, 20, 200, 2000 ppm) used. The hexane extract of *T. alexandri* showed very good antibacterial activity for many bacteria tested almost on par with ampicillin. except *K. pneumoniae*. The Zone of inhibition (in mm) were found to be 16 mm for *B. subtilis* and 15 mm

for both *E. faecalis* and *P. aeruginosa*, 14 mm for *P. vu*lgaris, 12 mm for *S. aureus* and 8 mm for *E. coli*, all at the concentration of 5000 ppm of hexane extract. Antibacterial activity from the body wall of several echinoderm species has been studied by Villasin and Pomory, (2000). Stabili et al., (1996) have been studied the antibacterial activity in the coelomocytes of the sea urchin *Paracentrotus lividus*. Antimicrobial activity has been found from the eggs of other marine invertebrates as well (Benkendorff et al. 2001; Haug et al., 2002) and both of these studies showed that at least some of the antibacterial compounds are not proteinaceous. In the FTIR study shows the presence of functional group such as chloroalkanes, bromoalkanes, iodoalkanes, alcohols groups, acids or aromatic ethers, methyl alkyl groups, 1° amines groups and ammonium ions which was responsible for the antimicrobial, hemolytic and cytotoxic effects. From the above study the sea urchin seems to be a promising source of heamolytic and cytotoxic compounds. Therefore the current study adds evidence to the presence of potent cytotoxic compounds in sea urchin of Cuddalore coast. Hence further purification and structural elucidation studies demands the description of cytotoxic lead molecules from these sea urchins.

5. Conclusions

Thus the current studies revealed the presence of potent bioactive compounds from sea urchin *T. toreumaticus*. Screening tactics followed by ecological knowledge of marine organisms are being increasing deployed in the investigation of novel bioactive compounds. Activities found in sea urchin *T. toreumaticus* showed promising results and the most suitable chemical and biological characterisation. Our preliminary result reveals that many of the marine organisms produce more or less structurally diverse secondary metabolites which could be of pharmaceutical interest. Large scale isolation will be necessary to obtain sufficient quantities of pure compounds for structure elucidation and further pharmacological evaluation.

Acknowledgments

Authors are thankful to the Director, Center of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai, Tamil Nadu, India for facilities provided.

References

Anderson, A. H., J. W. Mathieson and R. H. Thomson, (1969). Distribution of spinochrome pigments inechinoids. Comp. Biochem. Physiol., 28: 333-345.

Benkendorff, K., A. R. Davis and J. Bremner, (2001). Chemical defense in the egg masses of benthic invertebrates: an assessment of antibacterial activity in 39 mollusks and 4 polychaetes. J. Invertebr. Pathol., 78: 109-118.

Bradford, M. M., (1976). A rapid and sensitive method for the quantification of microgram quantities of protein using the principle of protein dye binding. Anal. Biochem., 72: 248-254.

Bragadeeswaran, S., K. Ganesan, N. Sri Kumaran, (2011). Hemolytic activities from ascidian *Polyclinum madrasensis* Sebestian, 1952 and *Phallusia nigra* Savigny, 1816 from Tuticorin coast of India. Asian J. Applied Sci., 4(6): 630-639.

Briskin, D., (2000). Medicinal Plants and Phyto medicines. Linking plant biochemistry and physiology to human health. Plant Physiology .,124: 507-514.

Carballeria, N. M., C. Cruz and A. Sostre, (1996). Identification of the novel 7-methyl-6 octadecenoic acid in *Holothuria mexicana*. J. Nat. Prod., 59(11): 1076-8.

Chantaro, P., S. Devahastin and N. Chiewchan, (2008). Production of antioxidant high dietary fiber powder from carrot peels. LWT-Food Science and Technology., 41: 1987-1994.

Clark, A. M and F. E. W. Rowe, (1971). Monograph of shallow water Indo west pacific echinoderms. British Museum (National History) Publication 690: 1-238.

Dubois, M, K. A. Giles, J. K. Hamilton, P. A. Rebors and F. Smith, (1956). Calorimetric method for determination of sugar and related substances. Anal. Chem., 28: 350 - 356.

Folch, I., M. Lees and G. S. H. Sloane, (1957). A simple method for the isolation and purification of total

lipids from animal tissue. J. Biol. Chem., 226: 497-509.

Haug, T., A. K. Kjuul, O. B. Styrvold, E. Sandsdalen, M. O. Olsen and K. Stensvag, (2002). Antibacterial activity in *Strongylocentrotus droebachiensis* (Echinoidea), *Cucumaria frondosa* (Holothuroidea) and *Asterias rubens* (Asteroidea). J. Invertebr. Pathol., 81: 94-102.

Lebedev, A.V., E. L. Levitskaya, E. V. Tikhonova and M. V. Ivanova, (2001). Antioxidant properties, autoxidation and mutagenic activity of echinochrome A compared with its etherified derivative. Biochem., 66: 885-893.

Li, C., T. Haug and K. Stensvag, (2010). Antimicrobial peptides in Echinoderms, ISJ 7: 132-140.

Mann, J. W. and J. R. Werntz, (1991). Catfish stings to the hand. Journal of Hand Surgery 16(2): 318- 321.

Meyer, B. N., N. R. Ferrigni, J. E. Putnam, L. B. Jacobsen, D. E. Nichols and J. L. McLaughlin (1982). Brine shrimp: a convenient general bioassay for active plant constituents. Planta Medica., 45: 31–34.

MINSAP, (1995). Guia metodologica para la investigacion fitoquimica en plantas medicinales. Ciudad de La Habana.

Mtolera, M. S. P. and A. K. Semesi, (1996). Antimicrobial activity of extracts from six green algae from Tanzania. Trends Marin Bot. East Afr. Region., 211-217.

National Committee for Clinical Laboratory Standards, (2006). Method for antifungal disk diffusion susceptibility testing in yeasts. Approved guideline M-44-A. National Committee for Clinical Laboratory Standards, M100 - S16, Wayne, PA: CLSI.

Pena-Cabrera, E. and L. S. Liebeskind, (2002). Squaric acid ester-based total synthesis of Echinochrome A. J. Org. Chem., 67: 1689-1691.

Proksch, P., W. E. G. Muller, (2006). Frontiers in Marine Biotechnology. Horizon Bioscience: Norfolk, UK.

Ridzwan, B. H., M. A. Kaswandi, Y. Azman, and M. Fuad, (1995). Screening for antibacterial agents in three species of sea cucumbers from coastal areas of Sabah. Gen. Pharmacol., 26: 1539-1543.

Rinehart, K. L., P. D. Shaw, L. S. Shield, J. B. Gloer, G. C. Harbour, M. E. S. Koker, D. Samain, R. E. Schwartz, A. A. Tymiak, E. G. Swynenberg, D.A. String Fellow, J. J. Vauva, J. H. Coats, G. E. Zurenko, S. L. Kuentzel, L. H. Li, G. J. Bakus, R. C. Brasca, L. L. Craft, D. N. Young and J. L. Connot (1981). Marine natural products as a source of antiviral, antimicrobial and antineoplastic agents. Pure. Appl. Chem., 53:795-817.

Service, M. and A.C. Wardlaw, (1984). Echinochrome-A as a bactericidal substance in the coelomic fluid of Echinus esculentus. Comp. Biochem. Physiol. B., 79, 161–165.

Sri Kumaran, N., S. Bragadeeswaran, T. Balasubramanian and V. K. Meenakshi, (2012). Bioactivity potential of extracts from ascidian *Lissoclinum fragile*, Afr. J. Pharmacy Pharmacol., 6 (25): 1854 -1859.

Sri Kumaran, N., S. Bragadeeswaran and V. K. Meenakshi (2011) Evaluation of antibacterial activity of crude extracts of ascidian *Didemnum psammathodes* Sluiter, 1895 against isolated human and fish pathogens. Asian Pacific Journal of Tropical Biomedicine 1(1) S1: 90-99.

Stabili, L., P. Pagliara and P. Roch, (1996). Antibacterial activity in the Coelomocytes of the sea urchin *Paracentrotus lividus*. Comp. Biochem. Physiol. B., 113 (3): 639-644.

Thangaraj, S. and S. Bragadeeswaran, (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. The Journal of Venomous Animals and Toxins including Tropical Diseases., 18(1): 53-61.

Uma, B. and R. Parvathavarthini, (2010). Antibacterial effect of hexane extract of sea urchin, *Temnopleurus alexandri* (Bell, 1884). Int. J. PharmTech Res., 2(3): 1677-1680.

Villasin, J. and C. M. Pomory, (2000). Antibacterial activity of extracts from the body wall of *Parastichopus parvimensis* (Echinodermata: Holothuroidea). Fish Shellfish Immunol.10: 465-467.