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Research Article

SCREENING ON ANTIMICROBIAL ACTIVITY OF MARINE GASTROPODS *BABYLONIA ZEYLANICA* (BRUGUIÈRE, 1789) AND *HARPA CONOIDALIS* (LAMARCK, 1822) FROM MUDASALODAI, SOUTHEAST COAST OF INDIA

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ABSTRACT

This aim of the study was to screen the presence of antimicrobial activities in two marine gastropods, such as *Babylonia zeylanica* and *Harpa conoidalis* against 20 human pathogens. In this assay ethanol and methanol were used for the extraction process. The antimicrobial activities were carrying out by standard disc diffusion method. The human bacterial pathogen *K. pneumonia* was showed maximum susceptibility (10.13±0.13mm inhibition zone) against ethanol extract of *B. zeylanica.* The ethanol extract of *H. conoidalis* shows maximum susceptibility (9.16±0.13mm inhibition zone) by *S. paratyphi*. The fungal pathogen *C. albicans* was showed maximum susceptibility (7.13±0.10mm inhibition zone) against methanol extract of *B. zeylanica*. The ethanol extract of *H. conoidalis* shows maximum susceptibility (4.03±0.05 inhibition zone) by *A. niger*. In Thin layer chromatography (TLC) of *B. Zeylanica* and *H. conoidalis* extracts were showed R_f value 0.92 and 0.87 respectively. The FTIR analysis represents the presents of aliphatic amiens, alkanes, alcohels and phenols compound of both gastropods extracts. The above observed result shows the gastropods extracts were rich in antimicrobial compounds. Further studies need for study the full structural components of the antimicrobial compounds from marine mollusc.

Keywords: Antimicrobial activity, Gastropods, *Babylonia zeylanica, Harpa conoidalis,* FT-IR, TLC

INTRODUCTION

The Drug resistance in microorganisms is one of the serious problems all over the countries. The microorganisms develop multidrug resistance by their peculiar pattern of adaptation behavior and problems of multi drug resistance in microorganisms are common in every field[1]. However new drug classes with novel mechanism of action will create effective therapy, at last for a period of time[2]. So there is an urgent need for the discovery of new and novel antimicrobial drugs to effectively eradicate the diseases producing microorganisms.

A wide variety of bioactive compounds are being isolated and characterized from the food that is derived from the marine environment, these natural compounds have been extracted from marine invertebrates, especially sponges, ascidians, bryozoans and mollusks and some of them are currently in clinical trials[3]. Most marine animal life is fixed to a substratum. It produces bioactive metabolites in response to ecological pressures such as competition for space, deterrence of predation and the ability to reproduce successfully. In a marine environment, where all surfaces are constantly exposed to the threat of surface colonisation, sessile organisms remain relatively free of biofouling. In addition, marine animals have strategies to defend themselves against foreign organisms, by production of secondary metabolites that repel them.

Marine invertebrates offer a source of potential antimicrobial drugs[4]. Antimicrobial peptides are important in the first line of the host defense system of many animal species. Studies of antimicrobial compounds of marine invertebrates may provide valuable information for new antibiotic discoveries and give new insights into bioactive compounds in marine molluscs. Molluscs, which are widely distributed throughout the world, have many representatives in the marine and estuarine ecosystem. Many bioactive compounds have been investigated predominantly for their antimicrobial, cytotoxic, anti-tumor and anti-inflammatory, antileukemic, antineoplastic and antiviral properties of molluscs[5,6,7,8,9]. Generally fewer extensive, investigations have been made of the antimicrobial proteins of molluscs groups and although whole body homogenates of some marine molluscs have been reported to contain a variety of antimicrobial compounds. In our continuous search of antimicrobial agents from natural source, this study designed to assess the antimicrobial properties of the tissue extracts of *Babylonia zeylanica* and *Harpa conoidalis* against human pathogens.

MATERIALS AND METHODS

Specimen collection and identification

Live specimens of Gastropods, *B. zeylanica* and *H. conoidalis* were collected from Mudasalodai landing centre (11º29′N 79º46′E), Southeast Coast of India. The collected fresh molluscs were preserved with ice and transported to the laboratory and identified by the standard literature of Subba Rao[10]. The animals were carefully removed from the shells.

Extraction

The extraction method was followed by Chellaram et al.[11]. The freshly collected mollusc tissues each 25 g in wet weight were soaked in methanol and ethanol separately and maintained for 3 days. The extracts were filtered through Whatman®No.1 filter paper and the solvents were concentrated by rotary evaporator (VC100A Lark Rotavapor® at 30°C) with reduced pressure to give a dark brown gummy mass. The resultant residues were stored at 4°C for further analysis.

Test microorganisms and microbial culture

The reference pathogens used to test antimicrobial assay were, the following gram-positive and gram-negative bacteria such as, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi, S. paratyphi*, *Klebsiella oxytoca, K. pneumoniae, Vibrio cholera, V. parahaemolyticus, Proteus vulgaris* and *P. mirabilis,* the fungal pathogens such as *Penicillium* sp., *Candida albicans*, *C. tropicalis, Aspergillus niger*, *A. flavus*, *Rhizopus* sp., *Trichophyton rubrum*, *Mucor* sp., *Alternaria alternata* and *Trichophyton mentagarophytes* were used. These microbes were collected from patients of Rajah Muthiah Medical College and Hospital, Annamalai University, Annamalai nagar, Chidambaram. Bacterial strains and fungal strains were cultivated maintained on nutrient agar and fungal agar slants at 4°C.

Antibacterial assay

Antibacterial activity was carried out by using standard disc diffusion method (Sri Kumaran et al.,[12]. The test cultures (bacteria 108 CFU/ml) were swabbed on top of the solidified media and allowed to dry for 10 min. The human bacteria were maintained on nutrient agar plates. The extracts of molluscs 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°C for 24 h. The susceptibility of the test organisms were determined by radius of the inhibition zone around each disc. The tetracycline discs (30 mg disc^{-1}) were used as a positive control and solvents discs were used as a negative control. All the extracts were tested with triplicate at attention of accurate results.

Antifungal assay

Antifungal activity was carried out by using the standard disc diffusion method by National Committee for Clinical Laboratory Standards National Committee for Clinical Laboratory Standards[13]. The extracts applied to 6 mm sterile discs in aliquots of 30 µL of solvent, allowed to dry at room temperature and placed on fungal agar plates seeded with microorganisms and incubated at 37ºC for 24 hrs. Zones of growth inhibition were measured in millimeters after incubation. The tetracycline discs (30 mg disc^{-1}) were used as a positive control and solvents discs were used as a negative control. All the extracts were tested triplicate at attention of accurate results.

Fourier Transform Infrared Spectroscopy (FT-IR) spectral analysis

The powered samples of *B. zeylanica* and *H. conoidalis* (10 mg) were mixed with 100 mg of dried potassium bromide (kbr) and compressed to prepare as a salt disc. The disc was then allow to read spectrophotometerically (Bio-Rad FTIR-40-model, USA). The frequencies of different components present in each sample were analyzed.

Thin layer chromatography (TLC)

Samples were analysed by TLC coupled to chemical tests for identification of different secondary metabolites according to MINSAP[14]. For analytical TLC, aluminum sheets (4x5 cm) coated with silica gel 60 F 254, were used. The chromatography was run in a chamber with n-Butanol: Acetic acid: Water (6:2:2v/v) as the mobile phase.

RESULTS

Antimicrobial activity

The crude extract from gastropods species *B. zeylanica* and *H. conoidalis* were screened against ten human pathogenic bacteria for testing their antimicrobial activities. The inhibition zones of methanol and ethanol extracts against the test organisms were given in (Table .1). The maximum inhibition zone (10.13±0.13 mm) was observed against *K. pneumonia* in ethanol extract of *B. zeylanica* and the minimum inhibition zone (1 mm) was observed by *V. cholerae* against same ethanol extract. In the case of *H. conoidalis,* maximum inhibition zone (9.16±0.13 mm) was observed against *S. paratyphi* in ethanol extract and minimum inhibition zone (1.03±0.05 mm) was noticed by *P. mirabilis* same extract. In fungal assay *C. albicans* shows high susceptibility (7.13±0.10 mm zone of inhibition) against methanol extract of *B. zeylanica* and lowest inhibition zone (1 mm) was noticed by *A. flavus* against ethanol extract. In the case of *H. conoidalis,* highest inhibition zone (4.03±0.05 mm) was noticed by *A. niger* against ethanol extract and lowest inhibition zone (1 mm) was noticed by *C. albicans* and *Mucor* sp. against same extract.

Table 1: Inhibition zone of crude extracts of *B.zeylonica* and *Harpa conoidalis* against human pathogens. (Mean ± SD)

Pathogens		Inhibition of zone(mm)(Mean±SD)				+ve Control	-ve Control
		Babylonia zeylanica		*Harpa conoidalis*			
		Methanol	Ethanol	Methanol	Ethanol		
Bacterial strains	*Proteus vulgaris*	9.1±0.08	10.1±0.08	3.83±0.25	2.2±0.08	11.06±0.05	0
	P. mirabilis	8.13±0.10	6.1±0.08	3.06±0.10	1.03±0.05	11.33±0.13	0
	Staphylococcus aureus	6.03±0.05	3.1±0.08	3.9±0.15	3.1±0.08	10.03±0.05	0
	Escherichia coli	3.1±0.08	4.2±0.17	2.03±0.05	3.16±0.25	13.06±0.10	0
	Salmonella typhi	3.16±0.13	1.33±0.25	2.1±0.08	2.2±0.30	11.1±0.15	0
	S. paratyphi	1.03±0.05	4.03±0.05	2	9.16±0.13	12.76±0.13	0
	Klebsiella oxytoca	5.13±0.13	3.16±0.18	-	2.1±0.08	14	0
	K. pneumonia	7.13±0.10	10.13±0.13	5.13±0.13	4.2±0.17	10.93±0.10	0
	V. cholerae	2.13±0.13	1	3.1±0.15	4.3±0.26	13.96±0.05	0
	V. parahaemolyticus	8.06±0.05	6.13±0.13	4.23±0.18	2.13±0.13	12.66±0.51	0
Fungal strains	*Candida tropicalis*	2.1±0.08	1.06±0.10	1.33±0.51	1.03±0.05	11	0
	C. albicans	7.13±0.10	2.03±0.05	2.03±0.05	1	11.46±0.10	0
	Trichophyton rubrum	1.03±0.05	2.16±0.13	3.1±0.08	2.1±0.15	12.03±0.05	0
	T. mentagaraphytes	3.16±0.13	1.03±0.05	-	1.86±0.10	14.03±0.05	0
	Aspergillus niger	4.13±0.10	5.06±0.10	4.03±0.05	2.96±0.05	13.2±0.30	0
	A. flavus	3.06±0.05	1	1.03±0.05	1.3±0.15	11.33±0.51	0
	Alternaria alternata	2.06±0.10	3.1±0.08	1.06±0.10	1.03±0.05	12	0
	Penicillium sp.	4.1±0.02	2	-	2.06±0.10	10.16±0.25	0
	Mucor sp.	1.16±0.25	2.1±0.08	3.06±0.10	1	10.9±0.15	0
	Rhizopus sp.	3.1±0.08	1.16±0.18	3.1±0.08	1.53±0.05	10.9±0.08	0

Table 2: FTIR frequency and functional group of gastropods

Frequency, cm^{-1}		Bond, functional group
Babylonia zeylanica	*Harpa conoidalis*	
665.44	671.23	C–Br stretch, Alkyl halides
1053.13	1039.63	C–N stretch, Aliphatic amies
1234.44	1240.23	
1409.96	1402.25	C–C stretch (in–ring), Aromatics
1543.05	1543.05	N–O asymmetric stretch, Nitro compounds
1649.14	1643.35	–C=C– stretch, Alkenes
2071.55	2079.26	-
-	2247.07	–C=C– stretch. Nitriles
2345.44		-
2937.59	2929.87	C–H stretch, Alkanes
3307.92	3427.51	O–H stretch, H–bonded, Alcohols,phenols
3398.57		
3782.41	3788.19	-
3963.72	3942.5	
3996.51		

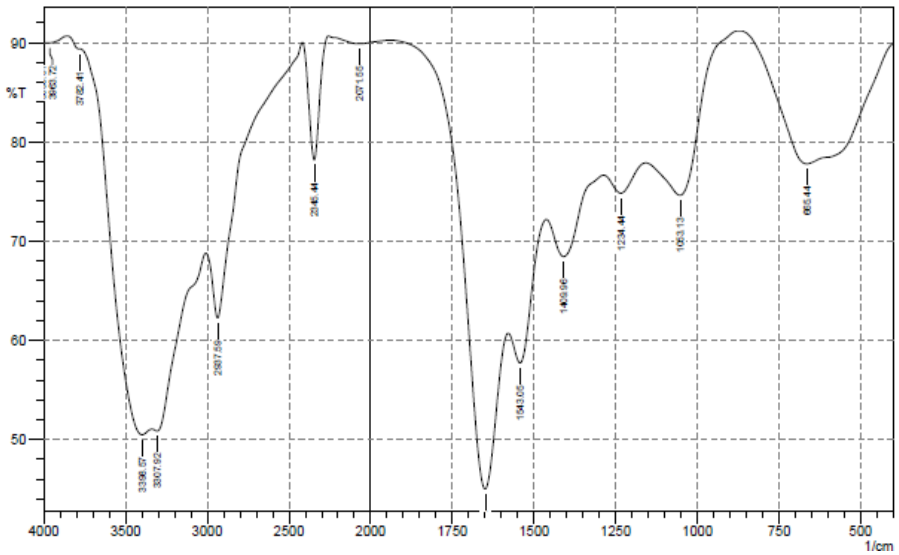

Fig. 1: The FTIR spectra of dried sample *B. zeylanica*

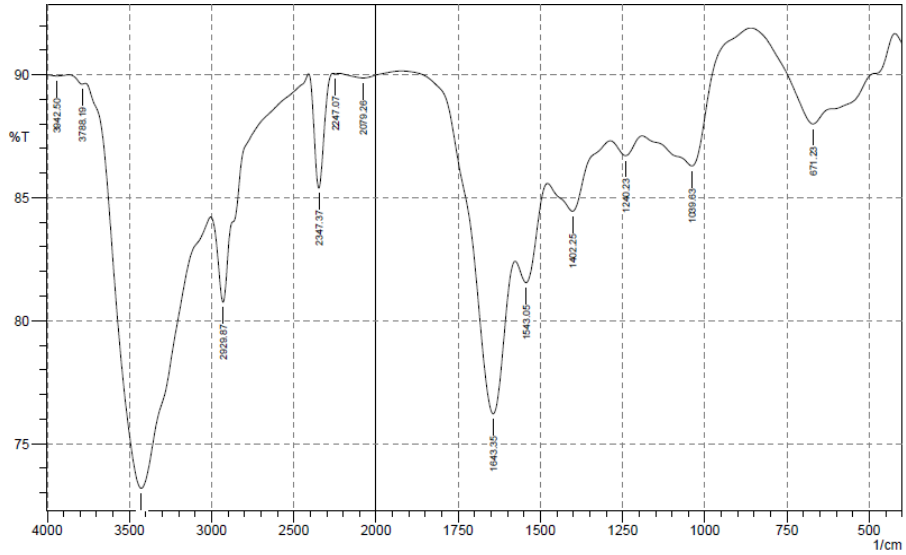

Fig. 2: The FTIR spectra of dried sample *H. conoidalis*

FTIR spectral analysis

The FTIR studies of these adsorbents helped for the identification of various forms of the compounds present in the molluscs extracts. The coupled vibrations are appreciable due to the availability of various constituents. Nevertheless, observed bands (in the range, 665.44-3996.51 cm^{-1}) have been tentatively assigned (Table. 2. Fig.1, 2)

Thin layer chromatography (TLC)

Thin layer chromatography on silica gel coated with the mobile phase n-Butanol: Acetic acid: Water (6:2:2v/v) showed an intensive spot (R_f =0.92 and 0.87) under UV light at 254 nm (Figure.3 shows that the organic phase from solvent extracts of two gastropods).

DISCUSSION

In recent years, great attention has been paid to the bioactivity of natural products because of their potential pharmacological utilization. Most of homeopathic medicines are either of plant or animal origin. Thousands of bioactive compounds identified in marine organisms reveal that sea creatures constitute a large reservoir for pharmacologically active drug recently reviewed[15]. Some of the molecules responsible for antimicrobial activities have been identified and characterized. Molluscs are widely used in world research institution for various studies, but only recently they have been recognized as potential sources of antibacterial and antifungal substances. Antibacterial and antiviral activities have been previously described in the hemolymph of several molluscan species such as, sea hares, sea slung, oysters, and mussels[16,17, 18,19,20,21,22]. The overall objective of the current study was capability of antibacterial and antifungal activity of ethanol and methanol extracts of the body tissue of two gastropods. In result of the present study clearly showed that the antibacterial activities maximum inhibition zone was observed in ethanol extract of *B. zeylanica* against *K. pneumonia.* In the case of *H. conoidalis* maximum inhibition zone was observed against ethanol extract by *S. paratyphi.* The antifungal activity in the highest inhibition zone was noticed against methanol extract of *B. zeylanica* by *C. albicans.* In the case of *H. conoidalis,* highest inhibition zone was noticed by *A. niger* against ethanol crude extract. As an early report has been made, the crude ethanol extracts of *B. spirata* showed good activities against *Pseudomonas aeruginosa*[23]. The methanol extract of *Hemifusus pugilinus* possessed the highest activity against *E.coli* and lowest activity was observed against *Klebsiella oxytoca*[24]. The methanolic extract of *chicoreus virgineus* and *C. ramous* experimentally analyzed and observed the broad

spectrum antimicrobial activities of body tissue extract[25]. The antibacterial activities of ethanol extracts of gastropod *B. spirata* and *Turbo brunneus* was observed maximum activities against *E. coli, K. pneumoniae, P. vulgaris* and *S. typhi* respectively[26]. The crude methanol extracts of *Cypraea errones* exhibited higher antibacterial and antifungal activities[5]. These results lend support to the present findings of the antimicrobial activity of *T. tissoti* and *B. spirata*[12]. From the above result express that the ethanol extracts of gastropods was shows predominant activity than methanol extracts. The knowledge of the self-defense mechanism of molluscs is extremely limited compared to that of vertebrates and arthropods.

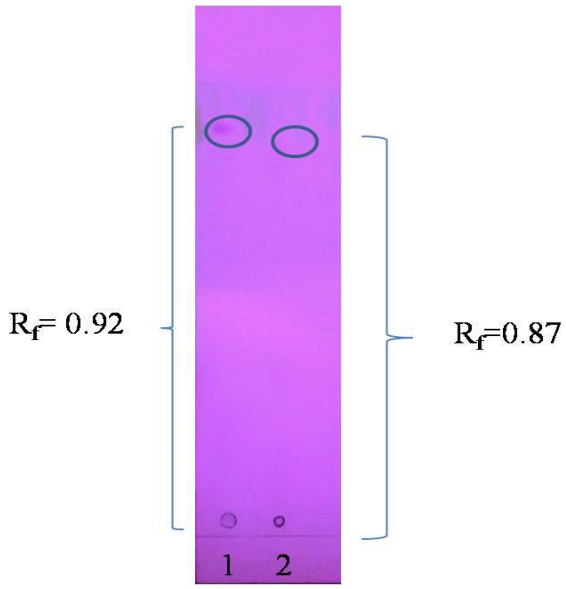

Fig. 3: TLC for solvent extract of gastropods.

1. *Babylonia zeylanica,* **2**. *Harpa conoidalis.*

In response to this selective pressure, a wide range of sessile marine organisms have evolved chemical defense mechanisms, which involve the production of secondary metabolites with antimicrobial activity[27]. The results of this study indicate that a wide range of invertebrates use chemical defense to protect their early stage embryos against bacterial infection[28]. The antimicrobial components in these egg masses could realistically play an ecological role in the prevention of microbial infection. Marine invertebrates rely solely on innate immune mechanisms that include both humoral and cellular responses. This gastropods species *B. zeylanica* and *H. conoidalis* are found in muddy bottom of the sea. The previous study of ascidian extracts for TLC shows the R_f value 0.2 and 0.3 with ethyl acetate: dichloromethane solvents (9:1 v/v), that the results alkaloids and peptides were clearly detected[29]. In result of the present study thin layer chromatography with solvents mixture of n-Butanol: Acetic acid: Water (6:2:2v/v) clearly showed the R_f value 0.92 and 0.87 respectively. The previous study of FTIR Showed 8 major peaks in lyopholyzed sample of *B. spirata*[23]. In the results of FTIR analysis reveals the presence of bioactive compound signals at different range (665.44-3996.51 cm^{-1}). Marine molluscs have been found to produce a great diversity of novel bioactive secondary metabolites and to be a potential source for new drug discovery. There has been a remarkable progress in the prevention; control and even eradication of infections diseases with improved hygiene and development of antimicrobial compounds and vaccines.

CONCLUSION

The present study revealed that the species of *B. zeylanica* and *H. conoidalis* showed antimicrobial activities against pathogenic microorganisms. This investigation was followed by the screening tactics based on the ecological knowledge of marine organisms being increasing deployed in the investigation of novel bioactive compounds. Antibacterial compounds from natural resources would be the alternative to overcome the resistance problems. It is promising that the tested gastropods species synthesis novel antibiotics for bacterial infections and fungal infections. Further investigations intending to purify these active compounds should be considered to clarify their chemical nature.

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REFERENCES

1. Amutha KB, Meenakshi VK, Senthamarai S. Evaluation of antimicrobial activity and antimitotic activities of biofouling marine ascidian extracts of Tuticorin coast. Int J Ph Sci 20110; 2(3): 750-758.
2. Allsup AE. New antibiotics discovery, novel screens, novel targets and impact of microbial genomics. Current opinion Micro 1998; 1: 530-534.
3. Proksch P, Edrada RA, Ebel R. Drug from the seas-current status and microbiological implications. Appl Microbiol Biotechnol 2002; 59: 125-134.
4. Bazes A, Silkina A, Douzenel P, Fay F, Kervarec N, Morin D, et al. Investigation of the antifouling constituents from the brown alga *Sargassum muticum* (Yendo) Fensholt. J Appl Phycol 2009; 21: 395-403.
5. Anand PT, Edward JKP. Antimicrobial activity in the tissue extracts of five species of cowries *Cyprea* sp. (Mollusca: Gastropoda) and an ascidian *Didemnum psammathodes* (Tunicata: Didemnidae). Indian J Mar Sci 2002; 25: 239-242.
6. Kamiya H, Muramoto K, Goto R, Sakai M. Purification and characterization of an antibacterial and antineoplastic protein secretion of a sea hare, *Aplysia juliana*. Toxicon 1989; 27:1269-1277.
7. Pettit GR, Kamano Y, Herald CI. The isolation and structure of a remarkable marine animal antineoplastic constituent, dolastatin - 10. J Am Chem Soc 1987; 109: 6883-6885.
8. Kisugi J, Ohye H, Kamiya H, Yamazaki M. Biopolymers from marine invertebrates. Mode of action of an antibacterial glycoprotein Aplysianin E, from eggs of a sea hare, *Aplysia kurodai*. Chem Pharma Bull 1989 b; 37(10): 2773-2776.
9. Rajaganapathi J, Thiyagarajan SP, Edward JKP. Study on Cephalopods ink for antiviral activity. J Exp Biol 2000; 38: 519-520.
10. Subba Rao NV. Indian sea shells (Part-1): Polyplacophora and Gastropoda. Rec Zool Sur India; 2003.
11. Chellaram CMEG, Patterson EJK. Antibacterial activity of the winged oyster *Pteria chinensis* (Pterioida: Pteridae). Indian J Mar Sci 2004; 33: 369- 372.
12. Sri Kumaran N, Bragadeeswaran S, Thangaraj S. Screening for antimicrobial activities of marine molluscs *Thais tissoti* (Petit, 1852) and *Babylonia spirata* (Linnaeus, 1758) against human, fish and biofilm pathogenic microorganisms. Afr J Microbiol Res 2011; 5(24): 4155-4161.
13. National Committee for Clinical Laboratory Standards. Method for antifungal disk diffusion susceptibility testing in yeasts. Approved guideline M-44-A. National Committee for Clinical Laboratory Standards, Wayne, PA; 2002.
14. MINSAP. Guia metodologica para la investigacion fitoquimica en plantas medicinales. Ciudad de La Habana. 1995.
15. Mayer AM, Rodriguez AD, Berlinck RG, Fusetani N. Marine pharmacology in 2007-8: Marine compounds with antibacterial, anticoagulant, antifungal, anti-inflammatory, antimalarial, antiprotozoal, antituberculosis, and antiviral activities; affecting the immune and nervous system, and other miscellaneous mechanisms of action. Comp Biochem Physiol 2011; 153C: 191-222.
16. Mitta G, Hubert F, Noel T, Roch P, Myticin, a novel cysteine-rich antimicrobial peptide isolated from haemocytes and plasma of the mussel *Mytilus galloprovincialis*. Eur J Biochem 1999a; 265: 71–78.
17. Nakamura T, Furunaka H, Miyata T, Tokunaga F, Muta T, Iwanaga S, **et al.**, A class of antimicrobial peptide from the hemocytes of the horseshoe crab, (*Tachypleus tridentatus*). Isolation and chemical structure. J Biol Chem 1988; 263: 16709-13713.

18. Zasloff M. Antimicrobial peptides of multicellular organisms. Nature 2002; 415: 389–395.
19. Gueguen Y, Herpin A, Aumelas A, Garnier J, Fievet J, Escoubas JM, et al., Characterization of a defensin from the oyster, *Crassostrea gigas*: recombinant production, folding, solution structure, antimicrobial activities, and gene expression. J Biol Chem 2006; 281: 313–323.
20. Maktoob A, Ronald HT, Handbook of natural products from marine invertebrates. Phyllum mollusca (Part. 1) Harwood academic publishers; 1997.
21. Olicard C, Renault T, Torhy C, Benmansour A, Bourgougnon N. Putative antiviral activity in hemolymph from adult Pacific oysters, *Crassostrea gigas*. Antiviral Res 2005; 66: 147–152.
22. Roch P, Yang Y, Toubiana M, Aumelas A. NMR structure of mussel mytilin, and antiviral–antibacterial activities of derived synthetic peptides. Dev Comp Immunol 2008; 32: 227–238.
23. Periyasamy N, Srinivasan M, Balakrishnan S. Antimicrobial activities of the tissue extracts of *Babylonia spirata* (Linnaeus, 1758) (Mollusca: Gastropoda) from Thazhanguda, southeast coast of India. Asian Pacific J Trop Bio 2012; 36-40.
24. Srinivasan G. Studies on antimicrobial properties of a gastropod *Hemifusus pugilinus* and a bivalve *Anadara granosa* (M.Sc Thesis). Annamalai University, Parangipettai; 2008, p. 27.
25. Santhana Ramasamy M, Murugan A. Potential antimicrobial activity of marine molluscs from tuticorin, southeast coast of India against 40 biofilm bacteria. J Shellfish Res 2005; 24(1):243-251.
26. Anand TP, Rajaganapathi J, Patterson Edward JK. 1997. Antibacterial activity of marine mollusc from Portonovo region. Indian J Mar Sci 26: 206-208.
27. Koh EGL. Do Scleractinian corals engage in chemical warfare against microbes? J Chem Ecol 1997; 23: 379–398.
28. Benkendorff K, Bremner JB, Davis AR. A putative role for the precursors of Tyrian Purple in the egg masses of the Australian Muricid, *Dicathais orbita*. J Chem Ecol 2000a; 26: 1037-1050.
29. Bragadeeswaran S, Ganesan K and Sri Kumaran N. Hemolytic activities from Ascidian, *Polyclinum madrasensis* Sebestian, 1952 and *Phallusia nigra* Savigny, 1816 from Tuticorin coast of India. Asian J Applied Sci 2011; 4(6): 630-639.