http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 16 (1): 38-43, 2013
ISSN 1028-8880 / DOI: 10.3923/pjbs.2013.38.43

Antimicrobial Activities of Two Edible Bivalves *M. meretrix* and *M. casta*

S. Sugesh and P. Mayavu
Centre for Advance Study in Marine Biology, Annamalai University,
Parangipettai-608502, Tamil Nadu, India

Abstract: The marine invertebrates become one of hot spot for the lead of antimicrobial compounds. Two species of commercially available and edible bivalves (*M. meretrix* and *M. casta*) were assayed for antimicrobial activity against 10 bacterial pathogens and 6 fungal pathogens and its biochemical composition. The bivalves were extracted with three different solvent systems respectively methanol, ethanol and acetic acid. All the three extracts of both the species *M. meretrix* and *M. casta* showed highest antibacterial activities against *S. aureus*, *E. coli*, *B. substillus*, *K. pneumonia*, *P. fleuroscence* and *V. cholera*. In present investigation the methanolic extract of the two bivalve species of *M. meretrix* and *M. casta* was showed inhibition activities against all pathogenic fungal forms. The two bivalve extracts showed high amounts of protein content, which made the variation up to 160-180 μg mg^{-1} (wet weight). Both samples had low amount of carbohydrates 4.77-5.77 μg mg^{-1} and lipids 0.11-0.17 μg mg^{-1}, respectively. The results of thin layer chromatography were revealed that presence of pink color spots it clearly indicates the presence of amino acid or peptides in bivalve's samples. Presuming that the antimicrobial compounds were proteins or peptides. In SDS-PAGE on 12% gel, the crude proteins *M. meretrix* and *M. casta* showed 5-6 bands ranging from 45-223 kDa. They represent potential pharmacological leads perhaps possessing novel and uncharacterized mechanisms of action that might ultimately benefit the ongoing global search for clinically useful antimicrobial agents.

Key words: Molluscs, bivalves, antibacterial activity, antifungal activity

INTRODUCTION

The ocean have a great biodiversity of flora and fauna estimated to over 5,00,000 species, more than double of the terrestrial species (Kamboj, 1999). The richness of the diversity offers a vast potential of novel chemicals that may be useful for finding new bioactive compounds with greater efficiency and specificity for treatment of many dread full human diseases (Bergmann and Feeney, 1951; Faulkner, 2000a, b; Da Rocha *et al*., 2001). Among the marine phylum, the invertebrates are the most potential sources for the pharmaceutical industry. Because of the sessile (sponges, tunicates, soft corals, etc.) animals which have lack of physical defenses, produce toxic chemicals to protect themselves in a very hostile environment and the chemical defenses have been developing in the oceans for thousands of years and resulted in nature's most toxic chemicals (Kathiresan *et al*., 2008).

The emergence of multiple-drug-resistant strains of microbial forms and the indiscriminate use of antibiotics highlight the urgent need for development of novel strategies to treat microbial infections. Marine organisms are the potential source for producing new innovative drugs. Recently, the marine invertebrates become one of hot spot for the lead of antimicrobial compounds. Bryostatin-1, a kind of macrocyclic lactone isolated from marine bryozoa *Bugula neritina* has already been in the secondary clinical trials and it found to be an excellent control over several types of cancers especially non-Hodgkin's lymphoma, chronic lymphocytic leukemia and acute lymphocytic leukemia (Kraft *et al*., 1996; Mohammad *et al*., 1998).

The discovery of new classes of antibiotic is necessary due to the increased incidence of multiple resistances among the pathogenic microorganisms to drugs that are currently in clinical use (Burgess *et al*., 1999). Peptides from marine invertebrates are promising candidates as new antibacterial agents due to their broad antimicrobial spectrum, highly selective toxicities and their difficulty for bacteria to develop resistant (Jimeno and Hidalgo, 2006; Tincu and Taylor, 2004). In the present study, two bivalve species (*M. meretrix* and *M. casta*) was screened to evaluate antimicrobial activities, already high percentage of bioactivities has been reported in marine bivalves (Chatterji *et al*., 2002, Lixin *et al*., 2005; Sumita *et al*., 2009). Two species of commercially available, edible bivalves (*M. meretrix* and *M. casta*) were assayed for antimicrobial activity.

Corresponding Author: S. Sugesh, CAS in Marine Biology, Annamalai University, Parangipettai-608502, Tamil Nadu, India Tel: (0) 04144- 243223, 243070

MATERIALS AND METHODS

Collection of specimens: The bivalves (*M. meretrix* and *M. casta*) were collected from Jan. 2009 to June 2010 in the mouth region of Vellar estuary and they were identified by using standard references (Satyamurthi, 1952) and also confirmed with zoological survey of India, Calcutta. The animals were not collected during the summer months to avoid stress related to diseases.

Preparation of bivalve extracts: The collected live animals were brought to the laboratory and the shells were broken and soft body, mantle fluid was removed. The extraction method was followed by Sumita *et al.* (2009). The collected materials were extracted in three different solvents such as ethanol, methanol and acetic acid (1:1). The extracts were homogenized at 15000 rpm for 15 min and the supernatant was filtered through No.2 filter paper. The filtrate was lyophilized to a powdered and used for antimicrobial studies.

Antimicrobial assay: The following bacterial and fungal pathogens were used for the antimicrobial assay: *E. coli*, *Salmonella typhi*, *S. paratyphi*, *Enterococcus faecalis*, *P. fluresence*, *Staphylococcus aureus*, *S. pyogenes*, *Proteus vulgaris*, *Klebsiella pnuemoniae* and *Vibrio cholera*) fungal pathogens are *Aspergillus flavus*, *A. fumigatus*, *Candida albicans*, *Mucur* sp., *Penicillium* sp. and *Cryptococcus pneuoformis* and these pathogenic forms were isolated from HIV+ve patients from Raja Muthiah Medical College, Annamalai University, Annamalai Nagar, Chidambaram. In vitro antimicrobial activity was determined by using the technique of Bauer *et al.* (1966).

Biochemical composition

Estimation of proteins: Proteins were estimated following the modified method of Bergmann and Feeney (1951) described by Peterson (1977). This method is applicable to soluble, membrane and conjugated proteins in dilute solutions, eliminating most interfering substances.

Estimation of carbohydrates: Carbohydrates were estimated following method of DuBois *et al.* (1956) was adapted.

Estimation of lipid: The chloroform-methanol extraction procedure of Folch *et al.* (1957) was used for extracting lipid from the soft body.

Thin layer chromatography and SDS-page: TLC profiling was done with both bivalve extracts with using two different solvent systems I and II. The solvent system I consist of methanol and chloroform (1:9) and solvent system II consist of combination of butanol, acetic acid and water (B:A:W) in the proportion of 5:1:4. The plates were showed pink color spots in both solvent systems when sprayed with ninhydrin solution. SDS-page was performed in 12% separating gels, according to the method of Laemmli (1970).

RESULTS

Antibacterial activities: The methanolic extract of bivalve *M. meretrix* showed maximum inhibition zone against *S. aureus* (10 mm), *E. coli* (10 mm) (Fig. 1) and minimum inhibition zone against *K. pneumonia* (2 mm). In ethanolic extract highest inhibition zone was observed in *E. coli* (12 mm), Likewise acetic acid extract of *M. meretrix* was showed maximum activities against *E. coli* (12 mm) and it showed minimum activities against *P. vulgaris*, *K. pneumonia* (3 mm).

In the crude methnolic extract of *M. casta* was showed highest inhibition of zone against *S. typhi* (12 mm). The *P. fluorescence*, *B. substillus* were showed highly resistant to crude methanolic extract. Ethanol extract of showed maximum zone of inhibition against *B. substillus, K. pneumonia* (12 mm). Acetic acid extract of *M. casta* was showed maximum activities against *E. coli* (13 mm), *Salmonella typhi* and *S. paratyphi* were showed resistant towards the acetic acid extract of *M. casta* (Fig. 2).

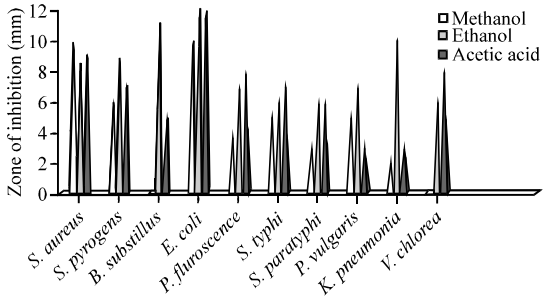

Fig. 1: Antibacterial activities of *M. meretrix*

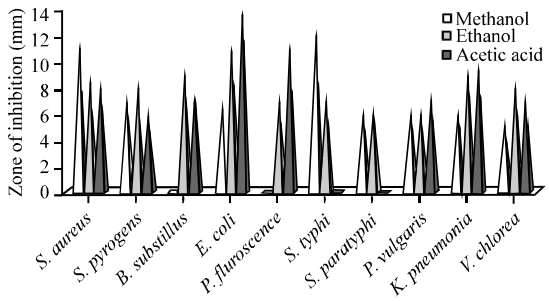

Fig. 2: Antibacterial activities of *M. casta*

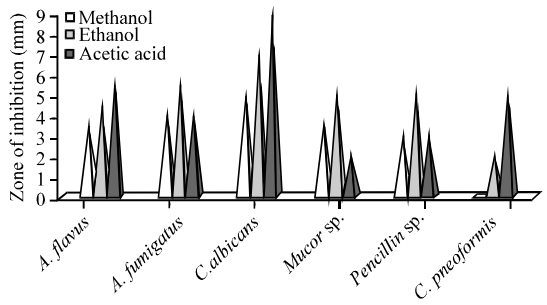

Fig. 3: Antifungal activities of *M. meretrix*

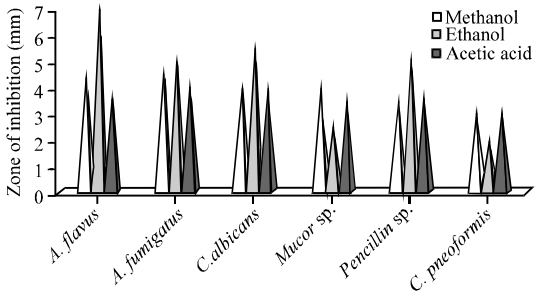

Fig. 4: Antifungal activities of *M. casta*

Table 1: Biochemical composition of marine bivalves

Test organism	Biochemical composition		
	Protein (μg mg^{-1})	Carbohydrates (μg mg^{-1})	Lipids (μg mg^{-1})
M. meretrix	165.4	4.77	0.11
M. casta	180	5.77	0.17

Antifungal activities: The methanolic extract of *M. meretrix* was showed maximum zone of inhibition against *C. albicans* (5 mm). *C. pneoformis* was highly resistant to the methanolic extract of *M. meretrix*. In ethanolic extract highest zone of inhibition was observed against *C. albicans* (7 mm) and the lowest activity against *Penicillium* sp. (2 mm). The acetic acid extract of *M. meretrix* was revealed that maximum zone of inhibition against *C. albicans* (9 mm) and it minimum activity against *Mucor* sp. (Fig. 3).

In crude methanolic extract of *M. casta* maximum zone of inhibition was observed against *A. flavus, A. fumigatus* (4.5 mm). The crude ethanolic extract of *M. casta* was showed highest zone of inhibition against *A. flavus* (5 mm) and it minimum activity against *Mucor* sp. (2.5 mm). The crude acetic acid extract of *M. casta* was showed maximum zone of clearance in *A. fumigates, C. albicans* (Fig. 4).

Biochemical composition: The results of bivalve species (protein, carbohydrates and lipid) were given in the Table 1. The bivalve *M. meretrix* were exhibited 165.4 μg mg^{-1} protein, 4.77 μg mg^{-1} carbohydrates and 0.11 μg mg^{-1} lipid. The *M. casta* was showed 180 μg mg^{-1}.

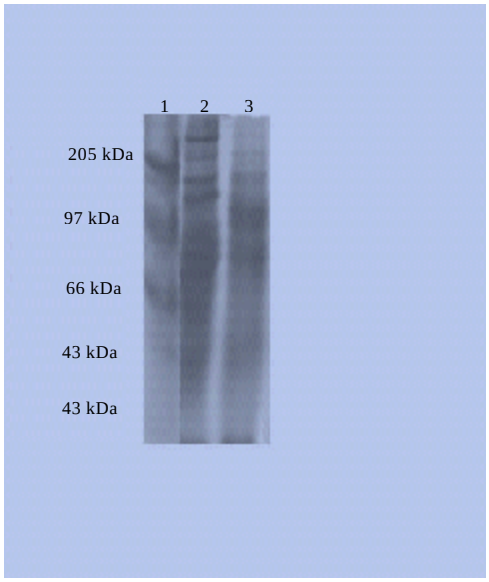

Fig. 5: SDS-page showed molecular weight of crude proteins, Lane 1: Standard markers, Lane 2: Crude extract of *M. meretrix*, Lane 3: Crude extract of *M. casta*

Thin layer chromatography (TLC): TLC profiling was done with both bivalve extracts with using two different solvent systems I and II. The solvent system I consist of methanol and chloroform (1:9) and solvent system II consist of combination of butanol, acetic acid and water (B:A:W) in the proportion of 5:1:4. The plates were showed pink color spots in both solvent systems when sprayed with ninhydrin solution. The pink color spot in TLC plate was clearly indicating the presence of amino acids and peptides.

SDS-PAGE: The crude extracts of both bivalve species were showed antimicrobial activities was subjected to SDS-PAGE for the estimation of the molecular weight of proteins present in it. For the estimation of molecular weights on crude extract of bivalve species protein using different standards such as, α-Macroglobulin (170 KDa), β-Galactosidase (116 KDa), Fructose-6-phosphate kinase (85.2 KDa), Glutamate dehydrogenase (55.6 KDa), Aldotase (39.2 KDa), Trios phosphate isomerase (26.6 KDa) and Trypsin inhibitor (20.1 KDa). In this present context, it revealed that both bivalve species have similar molecular weight proteins. Crude protein of *M. meretrix* yielded 6 bands ranging from 45 to 261 KDa with well defined bands at 45, 104, 149, 185, 223 and 261 KDa and in *M. casta* was showed 5 bands ranging from 50-218 KDa with well defined bands at 50, 86, 98, 125 and 218 (Fig. 5).

DISCUSSION

Bivalves possess several types of defense molecules including agglutinins and glycoproteins that have bactericidal activities (Johnshon, 1964; Arimoto and Tripp, 1977; Mori *et al*., 1980; Renwrantz and Stahmerge, 1983; Renwrantz *et al*., 1998). Study of modern medicine and pharmacology also showed that the flesh of *M. meretrix* has showed effects of antimutation (Yu *et al*., 1994), antitumor (Don *et al*., 1999) and immune promotion (Luo *et al*., 1996) activities. Several cystines rich AMPs were isolated from *M. galloprovincialis, M. edulis* (Mitta *et al*., 1999a; Charlet *et al*., 1996). The flesh of *M. meretrix* was used widely in India and China as a fisher folk medicine to treat the several liver diseases like Jaundice, Hepatitis-A and B (Wang *et al*., 2006). Likewise, the steroid extract of *M. meretrix* was inhibiting the cell growth and induction of G1-phase cell cycle arrest in hepatoma cells (Wu *et al*., 2006). Sumita *et al*. (2009) reported the antibacterial activities from edible bivalves *Perna viridis* and *M. casta*. The molluscan outer shells were found to have the ability to inhibit growth of pathogenic bacteria *E. coli, Staphylococcus aureus* and *Salmonella enteridis*, which cause food borne illness (Takama *et al*., 1999).

All the three extracts of both the species *M. meretrix* and *M. casta* showed highest antibacterial activities against *S. aureus, E. coli, B. substillus, K. pneumonia, P. fleuroscence* and *V. cholera*. The similar work made on other species of mollusc support of other workers, antibacterial activities of green mussel (*P. viridis*) and edible oyster (*Crassostrea madrasensis*) (Annamalai *et al*., 2007). Rajaganapathi (2001) reported the antimicrobial activities of marine mollusc and purification of anti-HIV protein. Henry *et al*. (1991) reported hemocytes of *Pinna nobilis* exhibited the antibacterial activity. Lectin isolated from heamolyph of the horse muscle *Modiolus modiolus* exhibited strong antibacterial activities against *V. anguillaram, V. salmonicida, V. viscosus, V. wondanis* and *V. ordalii* (Tunkijjanukij and Olafsen, 1998). Anand *et al*. (1997) reported that hypobranchial glands of *Chicoreous virgineous* and egg capsules of *Rapana rapiformis* were showed wide and spectral antibacterial activities against dreadful human pathogens. In present investigation the methanolic extract of the two bivalve species of *M. meretrix* and *M. casta* was showed inhibition activities against all pathogenic fungal forms. This finds supported by Rajaganapathi (2001) reported that methanolic extract of *A. rhombea* inhibit the growth of *Mucor* sp., *Penicillium* sp. and *A. flavus*. Charlet *et al*. (1996) reported that a peptide (Containing 12 cystines, about 6.2 kDa molecular weight) isolated from the blood of mussel (*M. edulis*) was showed antifungal activity. In the crude methanolic extract of *Cypraea errones* exhibited the antibacterial and antifungal activity against *S. aureus, S. pyogenes, A. niger* and *C. albicans* (Anand and Edward, 2002). Sumita *et al*. (2009) reported the antifungal activities two edible bivalve species *P. viridis* and *M. casta*. Gill tissue of the mussel of *M. edulis:* A new source of antimicrobial peptides. The saline extract of *M. meretrix* and *M. casta* was showed maximum zone of clearance against *A. fumigatus, A. flavus* and *C. albicans*. It was evidence by Jang *et al*. (2006) reported the antifungal activity of synthetic peptide derived from halocidin, from tunicate *Halocynthia aurantium* which showed the inhibition of *C. albicans*.

The two bivalve extracts showed high amount of protein content, which made the variation up 165 to 180 μg mg^{-1}. Both samples had low amount of carbohydrates 4.77-5.77 μg mg^{-1} and lipids 0.11 to 0.17 μg mg^{-1}, respectively. These values are similar to those reports for mantle and tissues of *M. casta*, which contained 190 μg mg mL^{-1} protein, 5.76 μg mg mL^{-1} carbohydrates and 0.15 μg mg mL^{-1} lipid respectively, (Sumita *et al*., 2009). Gopalakrishnan and Vijayavel (2009) reported nutritional composition of three estuarine bivalve's *P. viridis, Donax caneatus* and *M. meretrix*. Jayabal and Kalyani (1986) reported biochemical composition of *M .meretrix* from Bay of Bengal. Generally, bivalves are rich in nutrients, particularly proteins, fats and minerals. The Indian edible bivalves have protein (5-14%), fats (0.5-3%), calcium (0.04-1.84%) and phosphorus (0.1-0.2%) and iron (1-29 mg/100 g of the fresh weight).

The results of thin layer chromatography were revealed that presence of pink color spots it clearly indicating presence of amino acid or peptides in bivalve's samples. Presuming that the antimicrobial compounds was proteins or peptides. There are several reports are from the antimicrobial peptides has been reported from the heamocytes (Charlet *et al*., 1996; Mitta *et al*., 1999a, b), epithelial tissues (Marshall and Arenas, 2003; Iijima *et al*., 2003) and tissues gut and respiratory organs (Tincu and Taylor, 2004) of bivalve molluscs.

In SDS-PAGE on 12% gel, the crude proteins *M. meretrix* and *M. casta* showed 5-6 bands ranging from 45-223 KDa. Sumita *et al*. (2009) observed that unclear bands ranging from 14 and 29 KDa in marine bivalves *M. casta* and *P. viridis*. This Similar result was from *Perna canaliculus* containing the protein with molecular weight of 35 KDa (Scotti *et al*., 2001). Likewise. in *M. edulis* are containing histidine-rich glycoprotein (Nair and Robinson, 1999; Renwrantz

sand Stahmerge, 1983). Chandren *et al.* (2009) observed 9.7 kDa proteins in gill of estuarine bivalve *P. viridis*.

CONCLUSION

The present investigation concluded that *M. meretrix* and *M. casta* contain several factors with antimicrobial activities, in various organs and tissues. Thin layer chromatography results were revealed that proteinaceous compounds are responsible for antimicrobial activities. They represent potential pharmacological leads perhaps possessing novel and uncharacterized mechanisms of action that might ultimately benefit the ongoing global search for clinically useful antimicrobial agents.

REFERENCES

Anand, P.T. and J.K.P. Edward, 2002. 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., 31: 239-242.

Anand, T.P., J. Rajaganapathi and J.K. Patterson Edward, 1997. Antibacterial activity of marine mollusc from Portonovo region. Indian J. Mar. Sci., 26: 206-208.

Annamalai, N., R. Anburaj, S. Jayalakshmi and R. Thavasi, 2007. Antibacterial activities of green mussel (*Perna viridis*) and edible oyster (*Crassostrea madrasensis*). Res. J. Microbiol., 2: 978-982.

Arimoto, R. and M.R. Tripp, 1977. Characterization of a bacterial agglutinin in the hemolymph of the hard clam, *Mercenaria mercenaria*. J. Invertebrate Pathol., 30: 406-413.

Bauer, A.W., W.M. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 45: 493-496.

Bergmann, W. and R. Feeney, 1951. Contributions to the study of marine products. XXXII. The nucleosides of sponges.I. J. Org. Chem., 16: 981-987.

Burgess, J.G., E.M. Jordan, M. Bregu, A. Mearns-Spragg and K.G. Boyd, 1999. Microbial antagonism: A neglected avenue of natural products research. J. Biotechnol., 70: 27-32.

Chandren, B., G. Rameskumar and S. Ravichadren, 2009. Antimicrobial activity from the gill extraction of *Perna viridis* (Linnaues, 1758). Global. J. Biotech. Biochem., 4: 88-92.

Charlet, M., S. Chernysh, H. Philippe, C. Hetru, J.A. Hoffmann and P. Bulet, 1996. Innate immunity: Isolation of several cysteine-rich antimicrobial peptides from the blood of a mollusc, *Mytilus edulis*. J. Biol. Chem., 271: 21808-21813.

Chatterji, A., Z.A. Ansari, B.S. Ingole, M.A. Bichurina, M. Sovetova and Y.A. Boikov, 2002. Indian marine bivalves: Potential source of antiviral drugs. Curr. Sci., 82: 1279-1282.

Da Rocha, A.B., R.M. Lopes and G. Schwartsmann, 2001. Natural products in anticancer therapy. Curr. Opin. Pharmacol., 1: 364-369.

Don, C., F. Huang and L. Huang, 1999. An experimental study on the effects of meretrix polysaccharide on antineoplastic and immunorgulation. Chin. Mar. Drug, 2: 15-15.

DuBois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith, 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem., 28: 350-356.

Faulkner, D.J., 2000a. Highlights of marine natural products chemistry (1972-1999). Nat. Prod. Rep., 17: 1-6.

Faulkner, D.J., 2000b. Marine pharmacology. Anton. Leeuw., 77: 135-145.

Folch, J., M. Lees and G.H.S. Stanley, 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem., 226: 497-509.

Gopalakrishnan, S. and K. Vijayavel, 2009. Nutritional composition of three estuarine bivalve mussels, *Perna viridis*, *Donax cuneatus* and *Meretrix meretrix*. Int. J. Food Sci. Nutr., 60: 458-463.

Henry, M., N. Vincente and N. Houacho, 1991. Heamocycle characterization of marine bivalve e mollusc *Pinna nobilis*. Proceedings of the 8th National Congress of the French Malacological Society: Recent Aspects in Molluscan Biology, November 7-8, 1990, Brest, France, pp: 97-106.

Iijima, N., N. Tanimoto, Y. Emoto, Y. Morita, K. Uematsu, T. Murakami and T. Nakai, 2003. Purification and characterization of three isoforms of chrysophsin, a novel antimicrobial peptide in the gills of the red sea bream, *Chrysophrys major*. Eur. J. Biochem., 270: 675-686.

Jang, W.S., H.K. Kim, K.Y. Lee, S.A. Kim, Y.S. Han and I.H. Lee, 2006. Antifungal activity of synthetic peptides derived from Halocidin, antimicrobial peptide from the tunicate, *Halocynthia aurantium*. FEBS Lett., 580: 1490-1496.

Jayabal, R. and M. Kalyani, 1986. Biochemical studies in the hard clam *Meretrix meretrix* (L.) from Vellar Estuary, East Coast of India. Indian. J. Mar. Sci., 15: 63-64.

Jimeno, A. and M. Hidalgo, 2006. Pharmacogenomics of Epidermal Growth Factor Receptor (EGFR) tyrosine kinase inhibitors. Biochim. Biophys. Acta (BBA)-Rev. Cancer, 1766: 217-229.

Johnshon, H.M., 1964. Human blood group A_1 specific agglutinin of the butter clam *Saxidomus giganteus*. Science, 146: 548-549.

Kamboj, V.P., 1999. Bioactive Agents from the Ocean Biota. In: Ocean Science Trends and Future Direction, Somayajalu, B.L.K. (Ed.). Indian National Science Academy, New Delhi, India, pp: 197-227.

Kathiresan, K., M.A. Nabeel and S. Manivannan, 2008. Bioprospecting of marine organisms for novel bioactive compounds. J. Sci. Trans. Environ. Technovation, 1: 107-120.

Kraft, A.S., S. Woodley, G.R. Pettit, F. Gao, J.C. Coll and F. Wagner, 1996. Comparison of the antitumor activity of bryostatins 1, 5 and 8. Cancer Chemother. Pharmacol., 37: 271-278.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature, 227: 680-685.

Lixin, Z., F. Xiao and H. Lijun, 2005. Antitumor and immune regulation activities of the extracts of some Chinese marine invertebrates. Chin. J. Oceanol. Limnol., 23: 110-117.

Luo, Y., D. Wu and D. Ni, 1996. Pharmacologic effects of flesh of the clam *Meretrix meretrix* L. Chin. Mar. Drug, 15: 14-17.

Marshall, S.H. and G. Arenas, 2003. Antimicrobial peptides: A natural alternative to chemical antibiotics and a potential for applied biotechnology. Electron. J. Biotechnol., 6: 262-275.

Mitta, G., F. Hubert, T. Noel and P. Roch, 1999a. Myticin, a novel cysteine-rich antimicrobial peptide isolated from haemocytes and plasma of the mussel *Mytilus galloprovincialis*. Eur. J. Biochem., 265: 71-78.

Mitta, G., F. Vandenbulcke, F. Hubert and P. Roch, 1999b. Mussel defensins are synthesised and processed in granulocytes then released into the plasma after bacterial challenge. J. Cell Sci., 112: 4233-4242.

Mohammad, R.M., M.L. Varterasean, V.P. Almatchy, G.N. Hannoudi, G.R. Pettit and A. Al-Katib, 1998. Successful treatment of human chronic lymphocytic leukemia xenografts with combination biological agents auristatin PE and bryostatin 1. Clin. Cancer Res., 4: 1337-1343.

Mori, K., S. Itsukaichi, K. Murayama and T. Nomura, 1980. Activities of agglutinin and bactericidin in oyster tissues. Bull. Jpn. Soc. Fish. Sci., 46: 1385-1389.

Nair, P.S and W.E. Robinson, 1999. Purification and characterization of the histidine-rich glycoprotein that binds cadmium from the blood plasma of the bivalve *Mytilus edulis*. Arch. Biochem. Biophys., 366: 8-14.

Peterson, G.L., 1977. A simplification of the protein assay method of Lowry, which is more generally applicable. Anal. Biochem., 83: 346-356.

Rajaganapathi, J., 2001. Antimicrobial activities of marine molluscs and purification of antiHIV protein. Ph.D. Thesis. India.

Renwrantz, L. and A. Stahmerge, 1983. Opsonizing properties of an isolated hemolymph agglutinin and demonstration of lectin-like recognition molecules at the surface of heamocytes from *Mytilus edulis*. J. Comp. Physiol., 149: 535-546.

Renwrantz, L., W. Schmalmack and M. Steenbuck, 1998. Molecular size of native proteins of *Mytilus* serum which contains a dominant fraction with heavy metal-binding properties. Comp. Biochem. Physiol. Part A: Mol. Integr. Physiol., 121: 175-180.

Satyamurthi, T.S., 1952. Amphineura and gastropoda-I. Bull. Mad. Govt. Museum, 1: 220-229.

Scotti, P.D., S.C. Dearing, D.R. Greenwood and R.D. Newcomb, 2001. Pernin: A novel, self-aggregating haemolymph protein from the New Zealand green-lipped mussel, *Perna canaliculus* (Bivalvia: Mytilidae). Comp. Biochem. Physiol. Part B: Biochem. Mol. Biol., 128: 767-779.

Sumita, S., A. Chatterji and P. Das, 2009. Effect of different extraction procedures on antimicrobial activity of marine bivalves: A comparison. Pertanika J. Trop. Agric. Sci., 32: 7783.

Takama, K., T. Suzuki, H. Kaga, T. Takamura, Y. Taya, I. Shimono and S. Watanabe, 1999. Inhibition of the growth of food borne disease causing bacteria by calcined scallop shell powder. Bull. Fac. Fish. Hokkaido Univ., 50: 149-153.

Tincu, J.A. and S.W. Taylor, 2004. Antimicrobial peptides from marine invertebrate. Antimicrob. Agents Chemother., 48: 3645-3654.

Tunkijjanukij, S. and J.A. Olafsen, 1998. Sialic acid-binding lectin with antibacterial activity from the horse mussel: Further characterization and immunolocalization. Dev. Comp. Immunol., 22: 139-150.

Wang, G., B. Liu, B. Tang, T. Zhang and J. Xiang, 2006. Pharmacological and immunocytochemical investigation of the role of catecholamines on larval metamorphosis by β-adrenergic-like receptor in the bivalve *Meretrix meretrix*. Aquaculture, 258: 611-618.

Wu, T.H., R.L. Yang, L.P. Xie, H.Z. Wang and L. Chen *et al*., 2006. Inhibition of cell growth and induction of G1-phase cell cycle arrest in hepatoma cells by steroid extract from *Meretrix meretrix*. Cancer Lett., 23: 199-205.

Yu, Z.L., C.G. Dou and W.J. Jiang, 1994. Anti-mutation activity of *Meretrix meretrix*. Chin. Mar. Drug, 2: 34-34.