International Scholars Journals

Advanced Journal of Microbiology Research ISSN 2241-9837 Vol. 13 (6), pp. 001-006, June, 2019. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

In vitro evaluation of antimicrobial activity of methanolic extract from selected species of Cephalopods on clinical isolates

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Accepted 10 March, 2019

Bioactive substances from marine biota have been found useful as special tools in pharmacological and biomedical research. In the present study, the *in vitro* antimicrobial activity of crude methanolic extracts of six species of cephalopods (*Sepia kobiensis*, *Sepiella inermis*, *Sepioteuthis lessoniana*, *Octopus aegina*, *Octopus aerolatus*, *Octopus dollfusi*) from Cuddalore (Southeast coast of India) was studied. The antimicrobial activity was screened against 10 species of clinically isolated human pathogenic bacteria namely *Vibrio chlolerae, Pseudomonas aeruginosa, Klebsiella pnemoniae, Vibrio alginolyticus, Staphylococcus aureus, Vibrio parehaemolyticus, Streptococcus* sp., *Streptococcus pnemoniae, Salmonella* sp. and *Escherichia coli* and five fungal strains such as *Alternaria alternata, Candida tropicalis, Pencillium italicum, Fusarium equisetii* and *Candida albicans*. Different concentrations such as 25, 50, 75 and 100% were prepared and tested against the microbial strains for their inhibitory activities, using the disc diffusion method. The minimum inhibitory concentration (MIC) of methanolic extract of cephalopods ranged from 60 to 100 mg/ml. The results were discussed in the light of positive and negative control apart from the concentrations tested.

Keywords: Antimicrobial activity, cephalopods, minimum inhibitory concentration (MIC), pathogenic microorganisms.

INTRODUCTION

The Class: Cephalopoda includes nautilus, cuttlefishes, squids and octopods which are exclusively marine, varying in their form, size and nature (Voss and Williamson, 1971; Voss, 1973, 1977; Worms, 1983). They occupy littoral and benthic to pelagic environments of oceans. Cephalopods are important as a food resource as well as animal models in scientific investigations (Ngoile, 1987) and they are the store-house of many biologically important substances. There is an everlasting need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action due to the alarming increase that has been witnessed in the incidence of both new and re-emerging infectious diseases. A further big concern is the development of resistance to the antibiotics in current clinical use (Ilhan et al., 2007).

The first systematic search for antibiotics resulted in the discovery of actinomycetin from *Actinomycete* bacteria. In United States and Japan between 1953 and 1970 approximately 85% of the antibiotics were produced by *Actinomycetes,* 11% by fungi and 4% by bacteria (Reiner, 1982). Although antibiotics are life saving drugs but now-a-days, due to careless and promiscuous use of antibiotics, various pathogenic microbes are gaining resistance. Among marine invertebrates, cephalopods belong to a molluscan group comprising of 700 species in which bacterial associations have been known for a long time (Pierantoni, 1917; Bloodgood, 1977) which include the reproductive organs (accessory nidamental glands) of

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myopsids, sepiolids and sepiids (Kaufman et al., 1998; Grigioni et al., 2000; Pichon et al., 2005) and the light organ of sepiolids (McFall-Ngai and Ruby, 1991; Nishiguchi, 2002).

Marine invertebrates offer a good source of potential antimicrobial drugs (Bansemir et al., 2006; Mayer et al., 2007; Jayaraj et al., 2008). Discovered bioactive compounds in molluscs were identified essentially as peptide, depsipeptide, sterols, sesquiterpene, terpenes, polypropionates, nitrogenous compounds, macrolides, prostaglandins and fatty acid derivatives, miscellaneous compounds and alkaloids which presented specific types of activities (Maktoob and Ronald, 1997; Balcázar et al., 2006; Blunt et al., 2006). Studies on antimicrobial activity that lead to valuable information for new antibiotic discoveries and give new insights into bioactive compounds in aquacultured molluscs. In most of the publications concerning antimicrobial activity in molluscs, either single body compartments alone, like haemolymph and egg masses, or extracts of whole body have been tested for activity. Recently crustaceans (Haug et al., 2002a) and echinoderms (Haug et al., 2002b) are reported to possess antibacterial factors in different tissues. The antimicrobial activity of polysaccharides extracted from cephalopods such as *Sepia aculeata* and *Sepia brevimana* and heparin and heparin – like glycosaminoglycans (GAGs) from the cephalopod *Euprymna berryi* was reported against the human pathogenic microorganism (Shanmugam et al., 2008a; Shanmugam et al., 2008b). In the present investigation, an attempt has been made to screen the antibacterial and antifungal activity of the crude methanolic extract of selected cephalopods on some important human pathogens.

MATERIALS AND METHODS

Sampling and identification

Cephalopods such as, *S. kobiensis*, *S. inermis* (Cuttlefishes), *S. lessoniana* (Squid), *Octopus aegina*, *O. aerolatus* and *O. dollfusi* (Octopods), used in this study were obtained from Cuddalore landing centre (Latitude 10° 42'N; Longitude 79° 46'E) which is situated in the Southeast coast of India. The studies carried out by Voss (1973), Voss and Williamson (1971), Roper et al. (1984), Jothinayagam (1987) and Shanmugam et al. (2002) have been of considerable help on developing the identification keys and description which in most cases have also been corroborated with examination of actual specimen.

Preparation of extracts

Cephalopods were brought to laboratory; body tissues were removed, cut into small pieces and homogenized (REMI, RQ-127 A) and extracted with MeOH at room temperature for 24 - 48 h (Ely et al., 2004). Then the methanolic extract was centrifuged to collect the supernatant and concentrated under vacuum in a rotary evaporator (LARK, Model: VC-100A) at low temperature. The crude methanolic extract was assayed for antibacterial and antifungal activities using standard disc diffusion method.

Microbial cultures

Ten species of bacteria and five species of fungi were used as test organisms. (Bacterial strains- Gram- positive: *Staphylococcus aureus*, *Streptococcus* sp., *Streptococcus pneumoniae*; Gram-negative: *Escherichia coli, Vibrio cholerae*, *Vibrio alginolyticus*, *Vibrio parahaemolyticus, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Salmonella* sp.; Fungal strains- *Alternaria alternata, Candida tropicalis, Pencillium italicum, Fusarium equiseti* and *Candida albicans*). All the bacterial and fungal strains were clinical isolates, obtained from the Raja Muthaiah Medical College Hospital, Annamalai University, and Annamalai Nagar, India.

Inoculum preparation for bacteria

Nutrient broth was prepared and sterilized in an autoclave at 15 lbs pressure for 15 min. All the ten bacterial strains were individually inoculated in the sterilized nutrient broth and incubated at 37°C for 24 h. Mueller Hinton Agar (MHA, Himedia) was prepared, sterilized in an autoclave at 15lbs pressure for 15 min and poured into sterile petridishes and incubated at 37°C for 24 h. The 24 h old bacterial broth cultures were inoculated in the petridishes by using a sterile cotton swab.

Inoculum preparation for fungi

Czapek dox (Hi-media) broth was prepared and sterilized in autoclave at 15 lbs pressure for 15 min. Five fungal strains were inoculated in the broth and incubated at 37°C for 72 h. The sterilized Czapek dox agar was poured into sterile petridishes and incubated at 37°C for 3 days. The 72 h old fungal broth cultures were inoculated in the petridishes using a sterile cotton swab.

Disc diffusion method

Antibacterial and antifungal activity was determined following the method of (El-Masry et al., 2000). Briefly, a suspension of each tested microorganism was carefully mixed in the tube containing bacterial and fungal inoculums and media for bacterial and fungal were plated separately, respective strains were cotton swabbed on petridishes. Sterile antimicrobial disc (Hi-media) was impregnated with 50 µl of crude methanolic extract of the four concentrations tested. Positive control discs containing 50 µl of tetracycline (1 mg/ml) and negative control, 50 µl of methanol were used. The stocks for methanolic extracts were prepared in the concentration of 100 mg/ml. These impregnated discs were allowed to dry at laminar air flow chamber for 3 h, and were placed at the respective bacterial and fungal plates and incubated at 37°C for 24 h for bacteria and 72 h for fungi. The diameter (mm) of the growth inhibition halos produced by the methanolic extracts of cephalopods was examined. Result was calculated by measuring the zone of inhibition in millimeters. All the tests were performed in triplicates.

Determination of the minimum inhibitory concentration (MIC)

The methanolic extract of cephalopods which showed significant antimicrobial activity was selected for the determination of MIC followed by the method of Rajendran and Ramakrishnan (2009). A stock solution of 100 mg/ml was prepared and was serially diluted to obtain various ranges of concentrations between 20 and 100 mg/ml. 0.5 ml of each of the dilutions of different concentrations was transferred into sterile test tube containing 2.0 ml of nutrient broth. To the test tubes, 0.5 ml of test organism previously adjusted to a concentration of 10^5 cells/ml was then introduced. A set of test

Table 1. Antibacterial activity of methanolic extract of cephalopods.

Bacterial strains	*S. inermis* (%)				*S. kobiensis* (%)				*S. lessoniana* (%)				*O. aegina* (%)				*O. dollfusi* (%)				*O. aerolatus* (%)			
	25	50	75	100	25	50	75	100	25	50	75	100	25	50	75	100	25	50	75	100	25	50	75	100
Vibrio cholerae	-	+	+	++	-	-	-	-	-	++	++	++	-	+	+	++	-	-	+	+	-	+	+	++
P. aeruginosa	+	+	+	+	+	+	+	++	-	+	+	++	-	-	-	-	-	-	-	-	-	-	-	-
Klebsiella pneumoniae	+	+	++	++	-	+	+	++	-	-	+	++	-	-	+	++	-	-	-	-	-	-	-	-
V. alginolyticus	-	-	-	-	-	-	-	++	+	+	+	++	+	+	+	++	+	+	++	++	-	-	-	++
Staphylococcus aureus	+	+	++	++	-	-	-	-	-	+	++	++	-	-	-	+	-	+	++	++	+	+	+	++
V. parahaemolyticus	-	+	+	+	-	-	-	-	+	+	+	++	+	+	++	++	-	+	+	+	-	-	-	++
Streptococcus sp.	+	+	+	++	-	+	+	++	+	+	++	++	-	-	-	-	+	++	++	++	-	-		+
S. pneumoniae	-	+	+	+	-	-	-	++	-	-	-	-	-	+	+	++	-	-	+	+	-	-	-	-
Salmonella sp.	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	+	++	++	+++	-	-	+	++
E. coli	-	+	+	+	-	-	-	-	-	-	-	+	-	-	-	-	-	++	++	+++	-	-	-	-

(-) No activity, (+) Weak activity (7-10 mm dia.), (++) Good activity (11-15 mm dia.), (+++) Very good activity (above 16mm dia.) *The statistical singnicance: P values ≤0.05 (DMRT).

tubes containing broth alone was used as control. All the test tubes and control were then incubated at 37°C for 24 h. After the period of incubation, the tube containing the least concentration of extract showing no visible sign of growth was taken as the minimum inhibitory concentration.

Statistical analysis

Data on the inhibitory effect of methanolic extracts of cephalopods was analyzed by one-way analysis of variance (ANOVA) using SPSS-16 version software followed by Duncun's multiple range test (DMRT). P values ≤0.05 were considered as significant.

RESULTS

The methanolic extract of cephalopods showed antibacterial and antifungal activity against all pathogenic strains which were concentration-dependent i.e., the activity was higher in 100% concentration and lower in 25% concentration but activity was absent in negative control (Table 1).

In 100% concentration, the highest inhibition zone of 17 mm was observed against *E. coli* in *O. dollfusi* extract, 15 mm against *S. aureus* in *S. inermis* extract, 15 mm against *V. parahaemolyticus* in *S. lessoniana* extract, 15 mm against *V. parahaemolyticus* in *O. aegina* extract, 14 mm against *K. pnemoniae* in *S. kobiensis* extract and 13 mm against *S. aureus* in *O. aerolatus* extract. The lowest inhibition zone of 8 mm was observed against *Salmonella* sp. in *S. inermis* extract, 9 mm against *S. pnemoniae*, *E. coli*, *S. aureus* and *Streptococcus* sp. in *O. dollfusi*, *S. lessoniana*, *O. aegina* and *O. aerolatus* extract respectively and 11 mm against *S. pnemoniae* in *S. kobiensis* extract.

In 75% concentration, methanolic extract showed the maximum activity of 14 mm against *E. coli* in *O. dollfusi* extract, 13 mm against *V. parahaemolyticus* in *O. aegina* extract, 13 mm against *V. cholerae* in *S. lessoniana* extract, 12 mm against *K. pneumoniae* in *S. inermis* extract, 10 mm against *K. pneumoniae* in *S. kobiensis* extract and 10 mm was exhibited against *Salmonella* sp. in *O. aerolatus* extract. The minimum activity of 7 mm against *K. pneumoniae* in *O. aegina* extract, 8 mm against the *K. pneumoniae* in both *S. kobiensis* and *S. lessoniana* extract, 8 mm against *V. cholerae* and *Salmonella* sp. in *O. dollfusi* and *O. aerolatus* extract respectively.

In 50% concentration, the maximum activity of 12 mm was noticed against *E. coli* in *O. dollfusi* extract, 11 mm against *V. cholerae* in *S. lessoniana* extract, 10 mm against *V. parahaemolyticus* in *O. aegina* extract, 10 mm against *K. pneumoniae* in *S. inermis* extract, 8 mm against *K. pneumoniae* in *S. kobiensis* extract and 8 mm against *V. cholerae* in *O. aerolatus* extract. The minimum activity of 7 mm was recorded against *E. coli, P. aeruginosa*, *V. alginolyticus, V. cholerae* in *S. inermis, S. kobiensis, S. lessoniana* and *O. aegina* extract respectively and 7 mm was recorded against *S. aureus* in both *O. dollfusi* and *O. aerolatus* extracts.

In 25% concentration, maximum activity of 10 mm was recorded against *Salmonella* sp. in *O.*

Table 2. MIC of methanolic extracts of cephalopods against clinically isolated human pathogens.

Bacterial strains	*S. inermis* (mg/ml)					*S. lessoniana* (mg/ml)					*O. dollfusi* (mg/ml)				
	100	80	60	40	20	100	80	60	40	20	100	80	60	40	20
Vibrio cholerae	*	+	++	+++	+++	-	*	+	++	+++	*	+	++	+++	+++
P. aeruginosa	+	++	+++	+++	+++	*	+	++	++	+++	++	++	+++	+++	+++
Klebsiella pneumoniae	-	*	+	++	+++	*	+	++	+++	+++	++	++	+++	+++	+++
V. alginolyticus	++	+++	+++	+++	+++	*	+	++	++	+++	*	+	++	++	+++
Staphylococcus aureus	-	*	+	++	+++	-	*	+	++	+++	*	+	++	+++	+++
V. parahaemolyticus	+	+	++	+++	+++	*	+	++	+++	+++	+	++	+++	+++	+++
Streptococcus sp.	-	*	+	++	+++	-	*	+	++	+++	-	*	+	++	+++
S. pneumoniae	*	+	++	+++	+++	++	+++	+++	+++	+++	*	++	+++	+++	+++
Salmonella sp.	+	++	+++	+++	+++	+	++	+++	+++	+++	-	*	+	++	+++
E. coli	*	+	++	+++	+++	+	++	+++	+++	+++	-	-	*	+	+++

* MIC concentration, - No growth, + Cloudy solution (slight growth), ++ Turbid solution (strong growth), +++ Highly turbid solution (dense growth).

dollfusi extract, 8mm against *Streptococcus* sp. in *S. lessoniana* extract, against *V. parahaemolyticus* in *O. aegina* extract and against *K. pneumoniae* in *S. inermis* extract. The lowest activity of 7 mm against *V. alginolyticus* in both *O. aegina* and *O. dollfusi* extract, against *P. auruginosa* in *S. inermis* extract and the same level of inhibition found *V. parahaemolyticus* in *S. lessoniana* extract. But at the same time, no activity was recorded against all the fungal strains studied.

MIC of the active extract against the test organisms

The MIC results are given in Table 2. MIC values of *S. inermis* against bacterial strains such as *V. cholerae, K. pneumonia, S. aureus, Streptococcus* sp., *S. pneumonia* and *E. coli* showed 100, 80, 80, 80, 100 and 100 mg/ml respectively. In *S. lessoniana* the MIC for *V. cholerae, P. aeruginosa, K. pneumoniae, V. alginolyticus, S. aureus, V. parahaemolyticus* and *Streptococcus* sp. was recorded as 80, 100, 100, 100, 80, 100 and 80 mg/ml respectively. Whereas in *O. dollfusi* the MIC for *V. cholerae, V alginolyticus, S. aureus, Streptococcus* sp. *S. pneumonia, Salmonella* sp. and *E. coli* was recorded as 100, 100, 100, 80, 100, 80 and 60 mg/ml respectively.

DISCUSSION

The overall objective of the current study is to compare the ability of antibacterial and antifungal activity of crude methanolic extract of six species of cephalopods collected from the same habitat. In the present study the results clearly showed (Tables 1 and 2) that majority of extracts exhibited appreciable antimicrobial activity against most of the clinically isolated human pathogens. The effects of extracts were different for different bacterial strains. Interestingly the present study reported no antifungal activity for all methanolic extracts from the cephalopods studied.

In recent years, great attention has been paid to study the bioactivity of natural products due to their potential pharmacological utilization. The rationale of searching for drugs from marine environment stems from the fact that marine plants and animals have adapted to all sorts of habitats in the marine environment and these creatures are constantly under tremendous selection pressure including competition for space, predation, surface fouling and reproduction. Many of these organisms are showing antimicrobial properties. Although most of the antibacterial agents isolated from marine sources have not been active enough to compete with classicical anti-microbials obtained from microorganisms (Rinehart et al., 1981). However, majority of marine organisms are yet to be screened for discovering useful antibiotics.

Antibacterial activity has previously been described in a wide range of molluscan species such as oyster (*C. virginica*), mussel (*Mytilus edulis* and *Geukensia demissa*), muricid mollusks (*Dicathais orbita*) and sea hare (*Dolabella auricularia*) (Constantine et al., 1975; Gunthorpe

and Cameron, 1987; Prem Anand et al., 1997; Anderson and Beaven, 2001; Benkendorff et al., 2001). In most of the species studied, the haemolymph, egg masses or the whole body have been tested for activity. Antimicrobial peptides have been isolated and characterized from the haemocytes of *Mytilus edulis* (Charlet et al., 1996; Mitta et al., 2000a) and *M. galloprovincialis* (Hubert et al., 1996; Mitta et al., 1999; Mitta et al., 2000b), and from the seahare *Dolabella auricularia* (Iijima et al., 2003). In the present investigation the crude methanolic extract from the whole body tissue of the cephalopods was used to study the antimicrobial activity against selected human pathogens.

Prem Anand and Patterson Edward (2002) reported moderate antibacterial and antifungal activity from the extracts of various bivalves molluscs. Patterson and Murugan (2000) reported broad spectrum of antibacterial activity for aqueous ink extract of the cephalopods *L. duvaceli* and *S. pharaonis* against nine human pathogens.

EDTA extract (polysaccharides) of *D. sibogae* gladius reported 10 mm inhibition zone against *E. coli* and *K. pneumonia,* 9 mm inhibition zone against *S. aureus* and 7 mm against *S. typhii.* Whereas the EDTA extract of *L. duvauceli* showed only low activity i.e., 5 mm against *P. aeroginosa*, 4mm against *S. typhii* and *E. coli*. At the same time, the gladius extract of both the species showed no activity against *V. cholerae.* The polysaccharide extract from the gladius of *D. sibogae* recorded potent antibacterial activity against all the bacterial strains mentioned above and at the same time the polysaccharide extract of the *L. duvauceli* gladius recorded only low activity. The polysaccharides extracted from the gladius of *L. duvauceli* showed activity against the fungi such as *A. fumigatus*, *A. flavus* and *Rhizopus* sp; whereas gladius extract of *D. sibogae* reported activity against A*. fumigatus* and *Rhizopus* sp. only. But at the same time both the species showed no activity against *Candida* sp. at all the concentrations tested (Barwin vino, 2003). The cuttlebone extract (using EDTA) of *S. aculeata* and *S. brevimana* showed antibacterial activity against almost all the 9 pathogenic bacterial stains tested viz., *B. subtilis, E.coli*, *K. pnemoniae, S. aureus*, *V. parahaemolyticus, V. cholerae*, *S. typhii*. *P. aeroginosa* and *Shigella* sp*.* The activity was recorded in almost all the concentrations except in nega-tive control. The antifungal activity of cuttlebone extracts of *S. aculeata* and *S. brevimana* against four fungal stains such as *A. fumigatus, A. flavus*, *Candida* sp. and *Rhizopus* sp. showed the maximum activity of 100% and activity was found to be in an increasing order from the lower to higher concentration. On comparison the activity was higher in the cuttlebone extract of *S. aculeata* than *S. brevimana* (Shanmugam et al., 2008a).

Shanmugam et al. (2008b) reported that the crude and purified sample of Glycosaminoglycans (GAGs) from *E. berryi* showed activity against five pathogenic bacteria and four fungal strains. The activity was higher in 100% concentration and lower in 25% concentration; but activity was absent in negative control. The maximum antibacterial activity was shown in *Shigella* sp. (5 mm) for crude sample and *S. aureus* for purified sample. The minimum antibacterial activity showed (1.5 mm) against *E. coli* in crude sample. The maximum antifungal activity (5.5 mm) was observed against *C. albicans* and *A. fumigatus* in crude and purified sample, respectively. The minimum antifungal activity (0.5 mm) was recorded against *A. fumigatus* and *C. neofromans* in crude sample.

Although different species and experimental procedures were used in the different studies, they indicated the high frequency of detectable antimicrobial activity in marine molluscs. These results enforce the idea that cephalopods are a source to be considered in the discovery of new substances for drug development to control microbial diseases. In the present investigation highest inhibition was recorded in *Salmonella* sp. and *E. coli* against *O. dollfusi* extract. Kagoo and Ayyakkannu (1992) reported a broad spectral activity in the hypobranchial gland extract of *Chicoreus ramosus* against 10 bacterial strains. In the present study a wide spectrum of antibacterial activity has been recorded in almost all the extracts, (Table 1).

Conclusion

Cephalopods collected from Cuddalore landing centre, southeast coast of India showed potential antibacterial activity against human pathogenic bacterial strains. In the present evaluation good antimicrobial activity was seen in the extracts of *O. dollfusi*, *S. lessoniana* and *S. inermis* species, which indicates the presence of potent antimicrobial compounds in them. Further research on purification and characterization of these potentially active compounds, may pave the way for the development of potent antibacterial drugs.

ACKNOWLEDGEMENTS

Authors are thankful to the Director and Dean, CAS in Marine Biology, Faculty of Marine Sciences, and Annamalai University for providing necessary facilities. The authors (PR & NS) are also thankful to the Centre for Marine Living Resources and Ecology (CMLRE), Ministry of Earth Sciences, Cochin for the financial assistance.

REFERENCES

Anderson RS, Beaven AE (2001). Antibacterial activities of oyster (*Crassostrea virginica*) and mussel (*Mytilus edulis* and *Geukensia demissa*) plasma. Aquat. Living Res., 14: 343-349.

Balcázar JL, Blas Id, Ruiz-Zarzuela I, Cunningham D, Vendrell D, Múzquiz JL (2006). The role of probiotics in aquaculture. Vet. Microbiol., 114: 173-186.

Bansemir A, Blume M, Schröder S, Lindequist U (2006). Screening of cultivated seaweeds for antibacterial activity against fish

pathogenic bacteria. Aquaculture, 252: 79-84.

Barwin vino A (2003). Studies on cephalopos with reference to polysaccharides M. Phil., thesis, Annamalai University, Porto Novo India, p. 70.

Benkendorff K, Bremner JB, Davis AR (2001). Indole derivatives from the egg masses of Muricid molluscs. Molecules, 6: 70-78.

Bloodgood RA (1977). The squid accessory nidamental gland, ultrastructure and association with bacteria. Tissue and Cell, 9: 197-208.

Blunt JW, Copp BR, Munro MHG, Northcote PT, Prinsep MR (2006). Marine natural products. Nat. Prod. Rep., 23: 26-78.

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

Constantine GH, Catalfomo P, Chou C (1975). Antimicrobial activity of marine invertebrate extracts. Aquaculture, 5: 299-304.

Ely R, Tilvi Supriya C, Naik G (2004). Antimicrobial activity of marine organisms collected off the coast of South East India. J. Exp. Mar. Biol. Ecol., 309: 121-127.

El Masry HA, Fahmy HH, Abdelwahad ASH (2000). Synthesis and antimicrobial activity some new benzimidazole derivatives. Molecules, 5: 1429-1438.

Grigioni S, Boucher-Rodoni R, Demarta A, Tonolla M, Peduzzi R (2000). Phylogenetic characterization of bacterial symbionts in the accessory nidamental glands of the sepioid *S. officinalis* (Cephalopoda: Decapoda). Mar. Biol., 136: 217-222.

Gunthorpe L, Cameron AM (1987). Bioactive properties of extracts from Australian dorid nudibranchs. Mar. Biol., 94: 39-43.

Haug T, Kjuul AK, Stensvag K, Sandsdalen E, Styrvold OB (2002a). Antibacterial activity in four marine crustacean decapods. Fish Shellfish Immunol., 12: 371-385.

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

Hubert F, Knaap VD, Nobel W, Roch P (1996). Cytotoxic and antibacterial properties of *Mytilus galloprovincialis*, *Ostrea edulis* and *Crassostrea gigas* (bivalve molluscs) hemolymph. Aquat. Living Res., 9: 115-124.

Iijima R, Kisugi J, Yamazaki M (2003). A novel antimicrobial peptide from the sea hare *Dolabella auricularia*. Dev. Comp. Immunol., 27: 305-311.

Ilhan S, Savaroğlu F, Çolak F (2007). Antibacterial and Antifungal Activity of *Corchorus olitorius* L. (Molokhia) Extracts. Inter. J. Nat. Eng. Sci., 1(3): 59-61.

Jayaraj SS, Thiagarajan R, Arumugam M, Mullainadhan P (2008). Isolation, purification and characterization of [beta]-1,3-glucan binding protein from the plasma of marine mussel *Perna viridis*. Fish Shellfish Immunol., 24: 715-725.

Jothinayagam JT (1987). Cephalopods of the Madras coast, Zoological Survey of India, Tech. Monogra., 15: 85.

Kagoo IK, Ayyakkanu K (1992). Bioactive compounds from *Chocoreus ramosus* antibacterial activity *in vivo*. Phuket. Mar. Bio. Cent. Spec. Publ., 11: 147-150.

Kaufman MR, Ikeda Y, Patton C, Van Dykhuizen G, Epel D (1998). Bacterial symbionts colonize the accessory nidamental gland of the squid *Loligo opalescens* via horizontal transmission. Biol. Bull., 194: 36-43.

Maktoob A, Ronald HT (1997). Handbook of natural products from marine invertebrates. Phyllum: mollusca Part I. Harwood academic publishers, pp. 1-288.

Mayer AMS, Rodriguez AD, Berlinck RGS, Hamann MT (2007). Marine pharmacology in 2003-2004: marine compounds with anthelmintic antibacterial, anticoagulant, antifungal, anti-inflammatory, antimalarial, antiplatelet, antiprotozoal, antituberculosis, and antiviral activities; affecting the cardiovascular, immune and nervous systems, and other miscellaneous mechanisms of action. Com. Biochem. Physiol. C: Toxicol. Pharmacol., 145: 553-581.

McFall-Ngai MJ, Ruby EG (1991). Symbiotic recognition and subsequent morphogenesis as early events in an animal–bacterial mutualism. Science, 254: 1491-1494.

Mitta G, Hubert F, Nobel T, Roch P (1999). 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, Hubert F, Dyrynda EA, Boudry P, Roch P (2000a). Mytilin B and MGD2, two antimicrobial peptides of marine mussels: Gene structure and expression analysis. Dev. Comp. Immunol., 24: 381-393.

Mitta G, Vandenbulcke F, Hubert F, Salzet M, Roach P (2000b). Involvement of mytilins in mussel antimicrobial defense. J. Biol. Chem., 275: 12954-12962.

Ngoile MAK (1987). Fishery biology of the squid *Loligo forbesi* Steenstrup (Cephalopoda: Loliginidae) in Scottish waters. Ph.D thesis, University of Aberdeen, p. 218.

Nishiguchi MK (2002). Host-symbiont recognition in the environmentally transmitted sepiolid squid-Vibrio mutualism. Microb. Ecol., 44: 1-18.

Patterson EJK, Murugan A (2000). Screening of cephalopods for bioactivity. Phuket. Mar. Biol. Cen. Swpc. Pub., 21: 253-256.

Pichon D, Gaia V, Norman MD, Boucher-Rodoni R (2005). Phylogenetic diversity of epibiotic bacteria in the accessory nidamental glands of squids (Cephalopoda: Loliginidae and Idiosepiidae). Mar. Biol., 147(6): 1323-1332.

Pierantoni U (1917). Organsi luminosi, organi simbioticie ghiandola nidamental accessoria nei cefalopodi. Bull. Della. Soc. Nat. Napoli., 30: 30-36.

Prem Anand T, Patterson Edward JK (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., 25: 239-208.

Prem Anand T, Rajaganapathi J, Patterson Edward JK (1997). Antibacterial activity of marine molluscs from Porto novo region. Indian J. Mar. Sci., 26: 206-208.

Rajendran NK, Ramakrishnan J (2009). *In vitro* evaluation of antimicrobial activity of crude extracts of medicinal plants against multi drug resistant pathogens. Biyo. Bili. Ara□t. Der., 2(2): 97-101.

Reiner R (1982). Detection of antibiotic activity. In Antibiotics an introduction. Roche Scientific Services, Switzerland, 1: 21-25.

Rinehart KL, Shaw PD, Shield LS, Gloer JB, Harbour GC, Koker MES (1981). Marine natural products as a source of antiviral, antimicrobial and antineoplastic agents. Pure App. Chem., 53: 795-817.

Roper CFE, Sweeney MJ, Nauen CE (1984). Cephalopods of the world. Food and Agriculture Organization, Rome, Italy, 3: 277.

Shanmugam A, Purushothaman A, Sambasivam S, Vijayalakshmi S, Balasubramanian T (2002). Cephalopods of Parangipettai coast, East coast of India. Manogra, Centre of Advanced Study in Marine Biology, Annamalai University, p. 45.

Shanmugam A, Mahalakshmi TS, Barwin vino A (2008a). Antimicrobial Activity of Polysaccharides Isolated from the Cuttlebone of *Sepia aculeata* (Orbingy, 1848) and *Sepia brevimana* (Steenstrup, 1875): An approach to selected Antimicrobial Activity for Human pathogenic Microorganisms. J. Fish. Aqua. Sci., 3(5): 268-274.

Shanmugam A, Amalraj T, Palpandi C (2008b). Antimicrobial Activity of Sulfated Mucopolysaccharides [Heparin and Heparin – Like Glycosaminoglycans (GAGs)] from Cuttlefish *Euprymna berryi* Sasaki, 1929. Trends in App. Sci. Res., 3(1): 97-102.

Voss GL, Williamson GR (1971). Cephalopods of Hong Kong. Hong Kong: Hong Kong Government Press, p.138.

Voss GL (1973). Cephalopod resources of the world. FAO Fish Circ., 149: 1-175.

Voss GL (1977). Classification of recent cephalopods. Symp. Zool. Soc., Lond. 38: 575-579.

Worms J (1983). World fisheries for cephalopods. A synoptic overview. In: Caddy, JF (Eds), Advances in Assessment of World Cephalopod Resources. FAO Fish, Tech. Paper, pp. 231- 452.