WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 4, Issue 4, 798-807. Research Article ISSN 2277– 7105

IDENTIFICATION AND APPRAISAL OF CRUDE PROTEIN EXTRACTS OF INDIAN MARINE EDIBLE OYSTER *CRASSOSTREA GRYPHOIDES* FOR POTENTIAL BACTERICIDAL PROPERTY

P. Swapna and T. Ravinder Reddy*

Laboratory of Eco-physiology and toxicology, Dept. of Zoology, Kakatiya University, Warangal, Telangana, India.

Article Received on
28 Jan 2015,

Revised on 19 Feb 2015,
Accepted on 09 Mar 2015

***Correspondence for Author**
Prof.T. Ravinder Reddy
Dept. of Zoology,
Kakatiya university,
Warangal, Telangana,
India.

ABSTRACT

A wide range of bioactive substances are being isolated and characterized from the food that is derived from the marine environment, several with enormous assure for the treatment of various diseases. Marine organisms are a wealthy resource of structurally novel and biologically active metabolites. So far, frequent chemically exclusive compounds of marine origin with different biologically activity have been isolated and a number of them are under investigation and or are being developed as new pharmaceuticals. For the past two decades pharmaceutical industry has been relatively successful in overcoming problems due to single resistant determinants. In recent years, natural products from marine samples have a broad variety of biological activities and abundant therapeutic applications contain antiviral, antibacterial, antitumor activity and very different kinds of substances have been obtained. In general, the marine animals especially mollusks and their compounds constitute a practically unlimited resource of new active substances. Hence, the present study was carried out to determine the bactericidal activity of *Crassostrea gryphoides* protein against human pathogens. The edible Oyster *Crassostrea gryphoides* was collected from Kakinada seashore Andhra pradesh, India. Immediately it was extracted by using phosphate buffer at three different pH (4, 7 and 9) and all the extracts were screened against six different human pathogens such as *Vibrio furnissii*, *V. carchariae*, *Salmonella bongori* , *Shigella* sonnei, *enterococcus faecalis* and *Aeromonas hydrophila* by agar well diffusion assay. After 24 hrs of incubation the maximum inhibitory effect was observed against *Vibrio carchariae*, *Enterococcus faecalis* and *Aeromonas hydrophila* and the minimum inhibitory effect was observed against *Vibriocho furnissii*,

Salmonella bongori and *Shigella* sonnei respectively. Whereas checking the minimal inhibitory concentration (MIC), the crude protein extract of *Crassostrea gtyphoides* was inhibited the bacterial strains with the minimum inhibitory concentration of not less than 0.1ml (100¼). The molecular weight of the crude protein was found from 11.2 to 72.2 kDa and the total protein content of phosphate buffer crude extract of *Crassostrea gryphoides* was found to be 312 ¼g/ mg. From, the results, the work has suggested to use this commercially available and protein rich (bactericidal) oyster in therapeutics for the development of novel antibiotics against multiple drug resistance (MDR) pathogenic microbes.

KEYWORDS: *Crassostrea gryphoides*, pathogenic microbes. Therapeutics, novel antibiotics.

INTRODUCTION

The amino acid composition of peptides have amphipathicity, cationic charge and size allow them to attach to and insert into membrane bilayers to form pores by 'barrel-stave', 'carpet' or 'toroidal-pore' mechanisms. In fact several observations suggest that translocated peptides can alter cytoplasmic membrane septum formation, inhibit cell-wall synthesis, inhibit nucleic-acid synthesis, inhibit protein synthesis or inhibit enzymatic activity.[1] Consisting no more than a dozen aminoacids, rapidly produced and diffusible they seem ideal for fast and efficient defense against microbes.[2] A highly positive correlation between bacterial agglutination and bactericidal effect has been observed by lectins obtained from lobster, *Homarus americanus.* Other species possessing bacterial agglutinins in their body fluids include *P. bicarinatus, californiica , M. mercenerias* and the mollusc *Otala lac tea.*[3] It has also been reported that a purified lectin from Chinook salmon ova possesses antibacterial activity. Screening of antimicrobials from carribean sea animals and isolation of bactericidal proteins from the littoral mollusk cenchritis muricatus, Carlos Lopez-Abarrategui *et.al*. The growth of many drug resistant infectious disease and more upcoming disorders to human and animals are major problem in world wide.[4] Antimicrobial protein (peptides) is become as new antibacterial substance because of their bioactivity against resistance bacteria. The terrestrial resources have been greatly explored.[5]

Nowadays, the researchers are expecting the lead molecules and compounds from the new resources especially[6]. The Ocean covered more than 70% of the earth surface represent an enormous resource and from the past three to four decades many efforts have been committed for isolating various biologically active novel compounds from marine bio sources due to

their huge biodiversity and which offers a potential chemicals which can be useful for finding new bioactive compounds with greater effectiveness and specificity against human and animal pathogens.[7] There are, more than 12,000 natural products have been isolated from Marine algae, sponges, coelenterates, ascidians, echinoderms and bryozoans. Molluscs are a common prospect resource for the discovery of novel compounds for isolating bio active compounds to the pharmaceutical industry because most of the marine animals have lack of physical defenses, they produce toxic chemicals to protect themselves in a very hostile environment and still now most of them are unexplored.[8] Some studies have reported that, the bioactivity of the mollusks like *Aplysia* sp, *Phyllidae* sp, bivalves, gastropods, and their egg masses.[9]

Moreover, marine invertebrates are known to depend on innate immune mechanisms by interacting cellular and humoral components to protect against pathogens for their safe. Marine: the ultimate source of bioactives and drug metabolites. The *Crassostrea gryphoides* is one of the edible and commercially available species and the metabolites or bioactive compounds are still unexplored. Thus the present study was carried out to determine the bactericidal effect of commercially available and edible oyster *Crassostrea gryphoides* extract against six different human pathogens.

MATERIALSAND METHODS

Collection and Identification of *Crassostrea gryphoides* The edible Oyster ***Crassostrea gryphoides*** was ollected from Kakinada sea shore, Andhra Pradesh, India. The collected animals were identified by using standard manuals.

Extraction of Bactericidal peptides

The edible Oyster *Crassostrea gryphoides* was collected and transferred to the laboratory and washed with distilled water and the flesh samples were taken by breaking the shells. The peptides were prepared from the whole body tissue by phosphate buffer saline at three different pH (4, 7 and 9) by standard homogenization procedure. The homogenized mixtures were centrifuged at 4°C in 7500 rpm for 30 min. The supernatant was obtained, Freeze dried and stored at -20°C. The lyophilized crude extract was dissolved in 9.5 ml of phosphate buffer saline (PBS) at three different pH to obtain the partially purified protein by 85% ammonium sulfate precipitation and it was dialyzed. The dialyzed solution was freeze dried and stored at -20°C.

The lyophilized crude extract was dissolved in 9.5 ml of phosphate buffer saline (PBS) at three different pH to obtain the partially purified protein by 85% ammonium sulfate precipitation and it was dialyzed. The dialyzed solution was freeze dried and stored at -20°C. A stock solution of 2 mg/ml of lyophilized crude protein extract in sterilized PBS at three different pH(4, 7 and 9) was prepared for the further test.

Bactericidal activity The bactericidal potency of the crude protein extract of *Crassostrea gryphoides* was evaluated by adding 100¼l of each extract (water and phosphate buffer) against six different human pathogens such as *Vibrio furnissii, V. carchariae, Salmonella bongori, Shigella sonnei Enterococcus* and *Aeromonas hydrophila* by agar well diffusion assay. After the 24 hrs incubation, the zone of inhibition (ZOI) around the wells was measured. The assay was repeated in triplicate and the averages of the three were given as results.

MIC and MBC determination: The minimal inhibitory concentration (MIC) of the crude extract of *Crassostrea gryphoides* was determined by broth tube dilution assay. The *Crassostrea gryphoides* crude extract was prepared at various concentrations from 0.1ml to 0.5ml were determined for inhibitory level against all the human bacterial pathogens. The minimal inhibitory concentration (MIC) tubes were further carried out for Minimal Bactericidal concentration (MBC) evaluation using standard protocols. After 24 hrs of incubation period the loop full of cultures from the MIC and control tubes were transferred to the nutrient agar plates and the growth was monitored.

SDS PAGE Analysis

The proteins in the crude extract of *Crassostrea gryphoides* were purified and the molecular weight was confirmed by SDS PAGE analysis.

Estimation of protein concentration

The total protein concentration in the crude extract of *Crassostrea gryphoides* was estimated by the Lowry's method using BSA as standard.

RESULTS AND DISCUSSION

In general, the marine invertebrates such as cephalopods, gastropods, bivalves secrete or emit some substances which have a role in the chemical defenses and act against their predators. Some studies also proven that the compounds isolated from mollusks have exhibiting several

activities against human and animal pathogens. It is estimated, there are more than thousand new compounds has been categorized from marine invertebrates such as peptides, terpenes, polypropionates, nitrogenous compounds, polypeptides, macrolides, prostaglandins and fatty acid products, sterols and diverse compounds. Among the marine invertebrates the bivalves possess several types of defense molecules including agglutinins and glycoproteins which have bactericidal activities. In this present study, the edible Oyster *Crassostrea gryphoides* was collected from Kakinada sea shore, Andhra pradesh, India (Fig 1). Immediately it was extracted by using phosphate buffer at three different pH (4, 7 and 9) and all the extracts were screened against all the six different human pathogens such as Vibrio *furnissii, V. carchariae, Salmonella bongori*, *Shigella sonnnei*, *Aeromonas hydrophila* and *Enteroccus faecalis* by agar well diffusion assay and the zone around the wells were measured after incubation of 24 hrs (Table 1).

Table 1. Antimicrobial activity of *Crassostrea gryphoides* extract against human pathogens.

S. No	Human pathogens	Zone of Inhibition (mm) pH (4)	pH (7)	pH (9)
1	*Vibrio furnissii*	+	++	++
2	*V. carchariae*	++	+++	+
3	*Salmonella bongori*	+	++	++
4	*Shigella* sonnei	+	++	++
5	*Enterococcus faecalis*	++	+++	++
6	*Aeromonas hydrohila*	++	+++	+

Moreover, in all the tested human pathogenic bacteria were mostly inhibited by the crude protein extract pH 7 of *Crassostrea gryphoides* and the maximum inhibitory effect of 14mm was observed against *Vibrio carchariae*, *Aeromonas hydrophila* and *enterococcus faecalis* and the minimum inhibitory effect of 8mm was observed against *Vibrio furnissii*, *Salmonella bongori* and *Shigella* sonnei respectively (Fig 2). Similar results were also observed by previous studies. In their study they have reported that the edible bivalves *Perna viridis* and *M. casta* have the ability to inhibit growth of pathogenic bacteria *Staphylococcus aureus* and *Salmonella enteridis*, which cause food borne illness. Three different extracts of both *M. meretrix* and *M. casta* species against some pathogens, both the extracts have showed highest antibacterial activities against *B.substillus,K.pneumonia* and *P.fluroscence* respectively. Whereas checking the minimal inhibitory concentration (MIC) of the crude extract of *Crassostrea gryphoides* against all the human pathogenic bacteria at different dilutions (such as,0.1, 0.2, 0.3, 0.4 and 0.5ml) by broth tube dilution assay. The extract has inhibited the

bacterial strains with the minimum inhibitory concentration of not less than 0.1ml (100¼l) of the extract. The pathogenic bacterial strains such as *Vibrio carchariae*, *Aeromonas hydrophila* and *Enterococcus faecalis* were inhibited at 200¼l and remaining pathogenic bacterial strains such as, *Vibrio furnissii*, *Salmonella* bongori and *Shigella sonnei* were inhibited at 300 ¼l of *Crassostrea gryphoides* extract. Moreover the extracts also just inhibited the growth of many pathogenic bacteria at higher concentrations but not killed. The inhibitory and bactericidal concentration (MBC) remains same for extract of *Crassostrea gryphoides* against *Vibrio furnissii*, *V. carchariae* and *Salmonella bongori* .

Fig. 1. *Crassostrea gryphoides*

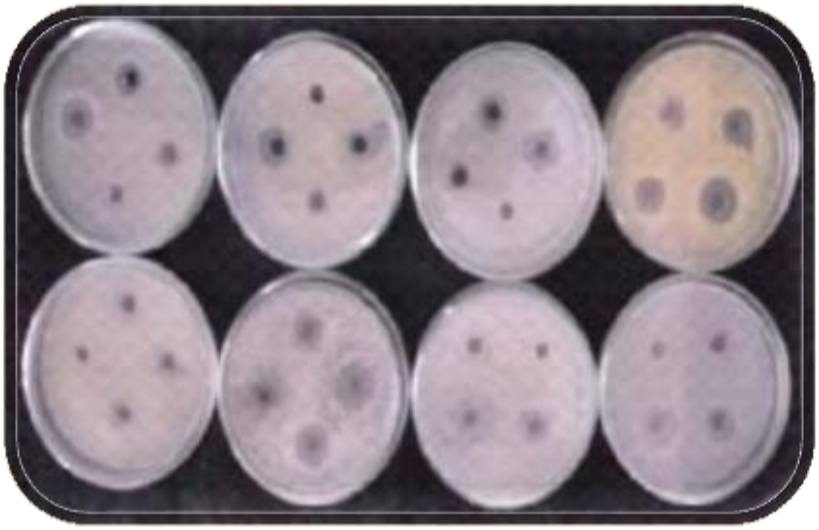

Fig. 2. Antibacterial activity of *Crassostrea gyrphoides is* extract against human pathogens.

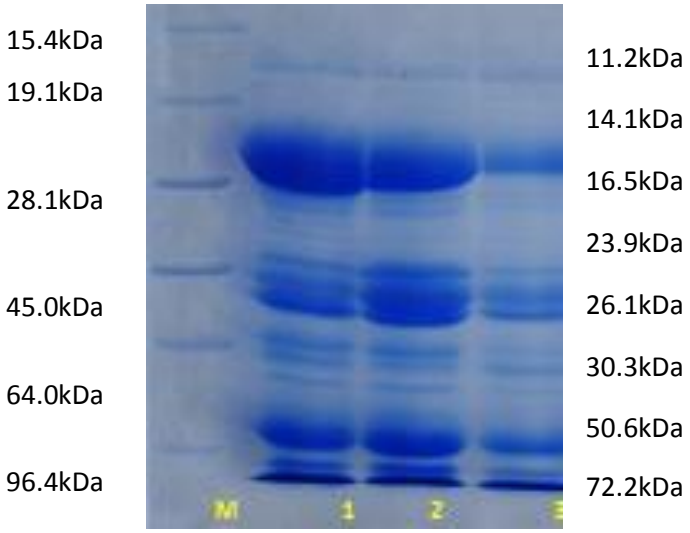

Fig. 3. Protein profile for the crude extract of *Crassostrea gryphoides* in SDS

(Table:1). Similar results were observed when inhibiting the growth of *Proteus vulgaris, Klebsiella pneumonia and Salmonella typhi* by using the crude protein extracts of *Pitar erycina* and *Donax cuneatus.* While analyzing the molecular weight of the crude protein extract of *Crassostrea gryphoides* using SDS-PAGE analysis with the marker range 15.4 to 96.4 kDa the results obtained with the separation of protein at 11.2, 14.1, 16.5, 23.9, 26.1 30.1, 50.6 and 72.2 kDa (Fig 3). Previous author have observed 5-6 bands ranging from 45 to 223 kDa from the extracts of *Meretrix meretrix* and *Meretrix casta*, similarly 35 kDa from *Perna canaliculus*24, 9.7 kDa from *Perna viridis* 25 and 3.5 Kda to 200 Kda from *Donax cuneatus and Pitar erycina* 16. The total protein (312 ¼g/ mg) in the extract of *Crassostrea gryphoides* was determined with the help of Lowry's method. The previous authors also reported, that the mantle and tissues of *Meretrix casta* has 190 ¼gmg-1 mL- 1 protein, 5.76 ¼g mg-1 mL-1 carbohydrates and 0.15 ¼g mg-1mL-1 lipid respectively.The nutritional composition of three estuarine bivalve's *Perna viridis, Donax caneatus* and *Meretrix meretrix* also resulted. In general, antimicrobial peptides (AMPs) are also act as major components of innate immune defence system in invertebrates, because the innate immunity is triggered immediately when the microbial infection occurs.

CONCLUSION

It can be concluded from our present investigation has suggested to use this antibacterial peptides from *Crassostrea gryphoides* for the development of novel antibiotics against to multiple drug resistance (MDR) pathogenic microbes.

ACKNOWLEDGEMENTS

The authors would like to acknowledge assistant professor Rajaiah, Department of Zoology, Jangoan for electrophoretic separation method and to the Head,Department of Zoology and biotechnology, Kakatiya University, Warangal, for providing laboratory facilities.

REFERENCES

1. Pistole TG. Interaction of Bacteria and Fungi with lectins and lectin like substances. Ann Rev Microbial 1981; 35: 85-112,
2. Scopes RK. Protein purification. Principles and practice (2nd edn.; Springer-verlag, New York). 1987; 302-306. .
3. Cruickshank R. Medical Microbiology. The Practice of Medical Microbiology (12th edn.); 1978.
4. Schauer R. Sialic acids and their roles as biological masks. Trends Biochem Sci 1985; 10: 357-360.
5. Chatterji.A, Ansari.Z.A.,et al.,. An extract obtained from marine animal having antiviral activities and process for its extraction. Indian patent, 2000; 159.
6. Carlos Lopez-Abarrategui et.al. Screening of antimicrobials from carribean sea animals and isolation of bactericidal proteins from the littoral mollusk cenchritis muricatus. Curr Microbiol; 2012; 64: 501-505.
7. S.Boobathy, T.T.Ajithkumar and Kathiresan. Isolation of Symbiotic Bacteria and Bioactive proteins from the marine sponge Callyspongia diffusa. Indian Journal of Biotechnology 2009; 8:272-275 .
8. S.Anbuselvi, C.Chellaram, S.Jonesh, L.Jayanthi and Edward. Bioactive Potential of Coral Associated Gastropod, Trochus tentorium of Gulf of Mannar, Southeastern India. J.Med.Sci. 2009; 9(5) 240-244.
9. M.I.Hoq, M.U.Seraj and Chowdary. Isolation and Characterisation of Antibacterial Peptides from Mud Crab, Scylla serrata. Pakistan Journal of Biological Sciences 2003; 6(15) 1345-1353.
10. Rajeev Kumar Jha, and Xu Zi-rong. Review Biomedical compounds from marine organisms. Marine Drugs; 2004; 2: 123-146.
11. Roshan Dinesh Yedery and Kudumula Venkata Rami Reddy. Purification and characterization of antibacterial proteins from granular hemocytes of Indian mud crab, Scylla serrata. Acta Biochimica Polonica, 2009; 56(1): 71-82.

12. Jirge Supriya S and Chaudhari Yogesh S. Marine: the ultimate source of bioactives and drug metabolites. International Journal of Research in Ayurveda & Pharmacy, 2010; 1(1): 55-62
13. Hong Young Yan. Harvesting drugs from the seas and how Taiwan could contribute to this effort. Changhua J Med 2004; 9:1-6.
14. Amparyup P, Kondo H, Hirono I, Aoki T, Tassanakajon A. Molecular cloning, genomic organization and recombinant expression of a crustin-like antimicrobial peptide from black tiger shrimp Penaeus monodon. Mol. Immunol. 2008; 45: 1085–1093.
15. Bachère E, Destoumieux D, Bulet P. Penaeidins, antimicrobial peptides of shrimp: a comparison with other effectors of innate immunity. Aquaculture 2000; 191: 71–88.
16. Bulet P, Stocklin R. Insect antimicrobial peptides: structures, properties and gene regulation. Protein Pept. Lett. 2005; 12: 3–11.
17. Carriel-Gomes MC, Kratz JM, Barracco MA, Bachere E, Barardi CR, Simoes CM. In vitro antiviral activity of antimicrobial peptides against herpes simplex virus 1, adenovirus, and rotavirus. Mem. Inst. Oswaldo Cruz 2007; 102: 469–472.
18. Schwartsmann G., Da Rocha AB., Berlinck JGS. and Jimeno J., Marine organisms as a source of new anticancer agents. Lancet Oncol., 2001. 2: 221– 225.
19. Aneiros A. and Garateix A., Bioactive peptides from marine sources: Pharmacological properties and isolation procedure. J. Chromatography B., 2004. 803: 41-53.
20. Anand PT, and 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.
21. Jayaseeli A., Anand TP. and Murugan A., Antibacterial activity of 4 bivalves from Gulf Mannar. Phuket. Mar. Biol. Cent. Publ., 2001. 25: 215-217.
22. Rajaganapathi J., Kathiresan K. and Sing TP., Purification Anti-HIV protein from purple fluid of the sea hare Bursatella leachii de Blainville. J. Mar. Biotechnol., 2000. 4: 447-453.
23. Lowry OH., Rosebrough NJ., Farr AL. and Randall RJ., Protein measurement with the folin phenol reagent. J Biol Chem., 1951. 193: 265-275.
24. Laemmli U.K., Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 1970. 227: 680-685.
25. Sivaperumal, P., Kamala K., Natarajan E. and Dilipan E., Antimicrobial peptide from crab haemolymph of Ocypoda macrocera (Milne Edwards1852) with reference to antioxidant activity: A case study. Int. J.Pharm. Pharm. Sci., 2013. 5 (2):719.

26. Becerro MA., Lopez NI., Turon X. and Uniz MJ., Antimicrobial activity and surface bacterial film in marine sponges. J. Exp. Mar. Biol. Ecol., 1994.179: 195-205.
27. Wright AE., Isolation of Marine Natural Products. In: Cannell RPJ, (ed.) Methods in Biotechnology, Natural Products Isolation, Humana Press Inc., New Jersey; 1998. 7: 305-408.
28. Metzer E., Agmon V., Andoren N. and Cohen D., Emergence of multidrug-resistant Salmonella enterica serotype Typhimurium phage-type DT104 among Salmonellae causing enteritis in Israel Epidemiol. Infect, 1998. 121: 555-559.