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Original Article

ANTIMICROBIAL POTENTIALOF HEMOLYMPH AND HEPATOPANCREAS OF*PORTUNUSSEGNIS*CRABS

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ABSTRACT

Objective: The potential of marine crabs as a source of bioactive products is largely unexplored.

Methods: Portunussegnis crabs were collected from the northern coast of Persian Gulf. Antimicrobial activities of hepatopancreas, hemolymph and proteins of hemplymph from *P. segnis* crabs against a range of 11 different bacterial and 6 fungal pathogen strains were examined. Two positive controls Amoxicillin and Penicillin were also used.

Results: The maximum(16 mm) and minimum (7.2 mm) activity was recorded from male hemolymph and male hepatopancreas against *Entrobacter* sp. and *Serratiamarcesences* respectively. Incomparison with bacterial pathogens, fungal pathogens showed more resistance to the extracts.

Conclusion: Results show that hemolymph and hepatopancreas of brachyuran crabs may have Antimicrobial activity.

Keywords: Crab, Hemolymph, Hepatopancreas, Antimicrobial.

INTRODUCTION

Antimicrobial peptide defense in crustacean has long been suspected. In 1972, bactericidal activities were observed in lobster *Homarusamericanus* plasma [4]and hepatopancreas[5]. Humoral immunity in marine invertebrate is characterized by antimicrobial agents present in the blood cells plasma, along with reactions such as hemolymph coagulation or melanization[6]. The circulating hemolymph in marine crustaceans contains biologically active substances such as complement, lectins, clotting factors and antimicrobial peptides [7]. Hence, the present investigation was taken up to study the antimicrobial activity of crab haemolymph and hepatopancreas extracts against 11 bacterial strains and 6 fungal strains.

MATERIALS AND METHODS

Collection of animals

The specimen was collected from the Bandar Abbas beach waters locatedin Hormozgan province in northern coast of Persian Gulf. Healthy male and female crabs at different stages of development were used throughout the experimental purposes and each crab was subjected to single blood collection.

Collection of Hemolymph

Haemolymph was collected by cutting each walking legs of the crab with a fine sterile scissor. To avoid haemocyte degranulation, haemolymph was collected in the presence of sodium citrate buffer (Merk), pH 4.6(2:1, V/V). The sample was dissolved in equal volume of physiological saline (%85, NaCl). Haemolymph was centrifuged at 10000 rpm for 10 minutes at 4^{o}C. The supernatant was collected by aspirating and stored at 4^{o}C until use[8].

Peptide purification by precipitation

The proteins of crudehaemolymph sample was precipitated by ammonium sulfate (%75) and stored at 4^{o}C overnight. The precipitate was collected by centrifuging at 15000 rpm for 20 minute at 4^{o}C and the pellet was re-suspended in acetate buffer(50 mM;pH 5.0), stored at 4^{o}C until use[8].

Preparation of hepatopancras extract

The hepatopancreas homogenized was filtered in vacuum through Whatman No. 4 filter paper. The residue obtained was then homogenized in two volumes of acetone for 3 min, and then the residue was air dried until the sample was dry and free of acetone odor. Defatted hepatopancreas powder obtained was stored at-20^{o}C until use. To prepare the hepetopancreas extract, the powder was suspended in 50 mMTris-Hcl, pH 7.5 containing 5 mM $CaCl_2$ referred to as starting buffer at a ratio of 1:10 (w/v) and stirred continuously at 4^{o}C for 3 h. The suspension was centrifuged at 12000 rpm for 10 min at 4^{o}C to remove the tissue debris, and the supernatant obtained was referred to as crude extract [9].

Microbial strains used

Antimicrobial activity of different extracts was determined against 11 bacterial strains viz: *Staphylococcus aureus* (PTCC 1189), *Bacillus subtilis* (PTCC 1156), *Bacillus cereus* (PTCC 1154),*Escherichia coli* (PTCC 1176),*Pseudomonas saeroginosa* (PTCC 1310),*Proteus mirabilis* (PTCC 1076), *Serattiamarcescens* (PTCC 1621),*Klebsiella pneumonia* (PTCC 1053),*Salmonellaentrica* (PTCC 1709), *Enterococcus faecalis*(PTTC 1237), *Entrobacter*sp. (PTCC 1291) and 6fungal strain:*Candida albicans* (PTCC 5027),*Fusariumsolani* (PTCC 5248), *Aspergillusniger* (PTCC 5223), *Fusariumsemytectum*, *Saprolegniaparasitica*and *Phytophthoracapsici*. These pathogenic strains were obtained from the Iranian Research Organization for Science and Technology (IROST).

Antibacterial Assay

In vitro antibacterial assay was carried out by disc diffusion technique [10]. Whatmann No. 1 filter paper disc with 6 mm diameter was impregnated with known amount test samples of the crude and ammonium sulphate precipitated crab haemolymph, hepatopancreas extract and positive control contained a standard antibiotic disc. The impregnated discs along with control (incorporated with sterile water) were kept at the center of Muller Hinton Agar(MHA) plates, seeded with test bacterial cultures. After incubation at room temperature (37^{o}C) for 24 h, antibacterial activity that expressed in term of diameter of zone of inhibition was measured and recorded.

Antifungal Assay

In vitro antifungal activity was determined using the technique of Bauer et al, 1996[10]. Six different species of fungal pathogen were inoculated by spread plate method using 0.1 ml of 72 hr old culture, maintained in Potato Dsextros Broth (PDB). Whatman No.1 filter

paper 6 mm disc impregnated with test samples of the crude and ammonium sulphate precipitated crab haemolymph, hepatopancreas extract and positive control contained a standard antibiotic disc. Control disc was placed on the Potato Dextros Agar (PDA) plates. After incubation at 30° C for 5-7 days, antifungal activity was measured in term of diameter of zone (including the disc within) in mm (14).

Result

The zone of inhibition in bacterial and fungal strains against different extracts is shown in figures 1to6. The male pure haemolymph showed a strong effect, more than other extracts, on bacterial and fungal strains. Among the tested samples maximum activity (16 mm) and (14.7 mm) was recorded against *Entrobacter* sp. and *S. entrica* from male haemolymph and protein precipitated from hemolymph respectively (fig. 1 and 2). Minimum activity (8 mm) and (7.2 mm) was recorded against *S. parasitica* and *Ph. Capcisi* from male hepatopancreas(fig.4).

Fungal pathogens showed more resistance than bacterial pathogens to the tested extracts. The haemolymph showed a significant bactericidal activity with regard to the Gram positive as well as Gram negative bacteria. Interestingly, *S. aureus*, *K. pneumonia* and *Ph. Capsici* were susceptible to all the extracts. Surprisingly, *F. solani* showed total resistance (no zone) (fig. 4). The resistance of *F. solani* needs further research to confirm the mechanisms involved. The haemolymph protein precipitated of crab didn't have good antimicrobial activity against the tested strains (fig. 5 and 6).

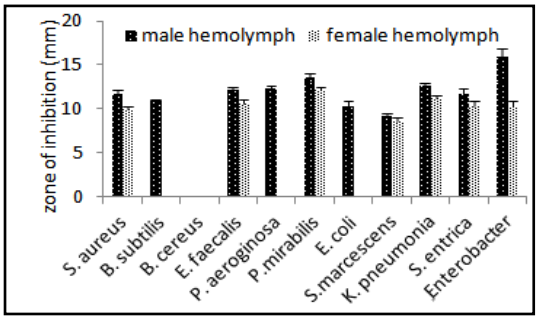

Fig. 1: Antibacterial activities of *p.segnis* male and Female Haemolymph (mean±SD)

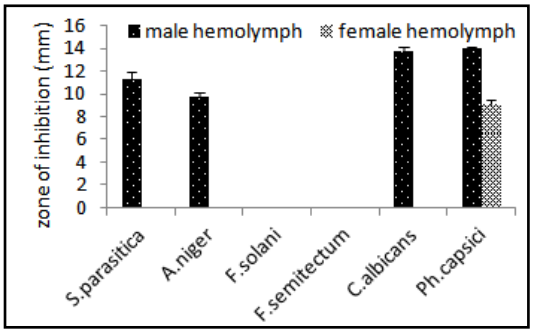

Fig. 2: Antifungal activities of *p.segnis* male and female haemolymph (mean±SD)

DISCUSSION

In the recent years, great attention has been paid to study the bioactivity of natural products due to their potential pharmacological utilization. In the present study, crabhaemolymph and hepatopancreas showed antimicrobial activity against different bacterial strains of both gram positive and gram negative bacteria and pathogenic fungal strains. Antimicrobial activity has been reported earlier in the haemolymph of the blue crab *Callinectessapidus* [11], mud crab *Scylla serrata*[12], *Ocypodemacrocera* [1] and *Carcinusmaenas*[13].

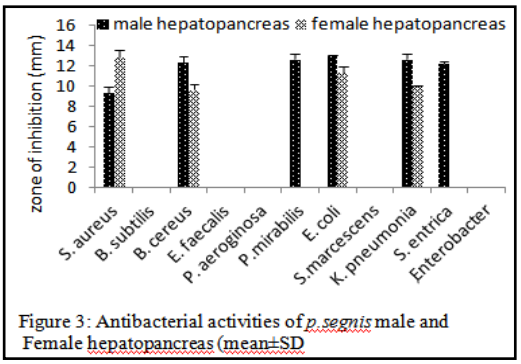

Figure 3: Antibacterial activities of *p.segnis* male and Female hepatopancreas (mean±SD

Fig. 3: Antibacterial activities of *p.segnis* male and female hepatopancreas (mean±SD)

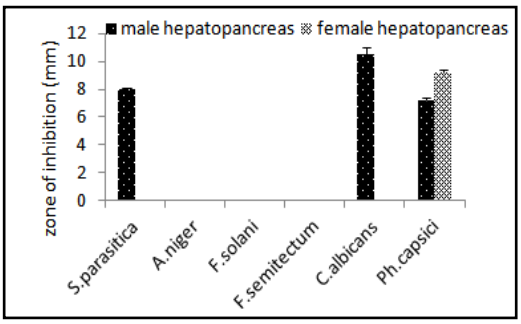

Fig. 4: Antifungal activities of *p.segnis* male and female haemolymph (mean±SD)

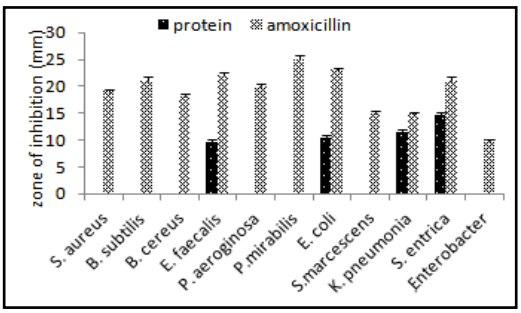

Fig. 5: Antibacterial activities of *p.segnis* haemolymph Protein and amoxicillin (mean±SD)

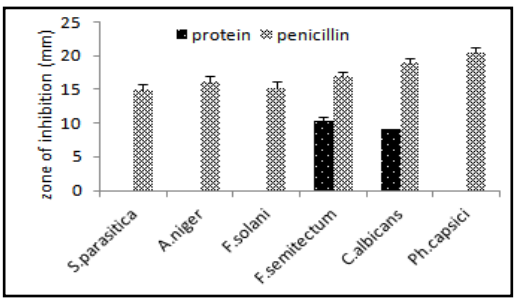

Fig. 5: Antibacterial activities of *p.segnis* haemolymph Protein and Penicillin (mean±SD)

But antimicrobialactivities of*P. segnis* crabs have not been reported earlier. Similar resultswere observed with the haemolymph of some crustaceans against clinical pathogens [3, 5, 14,15]. Nakamura et al., 1992 obtained same results from *Tachypleustridentatus*and Gudmundsson et al., 1991 in *Hyalophoracecropia*[16,17]. These results indicate that crabs have developed a variety of defense molecules in haemolymph against pathogenic microorganisms and

the kind of dominating vary among the different species [1]. Recently, marine peptides have opened a new perspective for pharmaceutical developments [2]. Crabs are the wonderful resource of antimicrobial proteins with awide range of antimicrobial properties which is highly supported in the haemolymph study of *Charybdis lucifera*[4], shore crabs (*Carcinusmaenas*) [18] and haemolymph of penaeid shrimp [19].

The concentration of protein in the haemolymph shows wide interspecific variation among the brachyuran crabs. Hemocytes might be the site of production and storage for these antimicrobial peptides. Decapods hemocytes are known to contain several immune effectors and they play a major role in the cellular and humoral defense mechanisms of the host [20]. It is believed that circulating hemocytes are playing an important role in the innate immune of invertebrates, including being the storage reservoir of several immune components, such as lectins, coagulation factors andproteas inhibitors [12]. It has been observed that in various invertebrate species, the hemolymph elicit the synthesis of a number of antimicrobial peptides and proteins after bacterial injection[21]. As the haemolymph showed antimicrobial activity, it offers to suggest that broad spectrum of antibacterial peptides were secreted in response to immunization[12]. Protein precipitated showed lesser inhibitory to fungal pathogens compared to bacteria pathogens. Many studies reported antifungal activity of hemolymphproteins[22,23]. Among the crude and purified haemolymph samples maximum activity was noticed in the crude extracts than purified. Maximum inhibition of crude sample is the common phenomena because the preservative and buffer mixture in the crab haemolymph will also some time inhibit the growth of bacteria strains [24]. The antimicrobial activity might also be due to factors of innate immune system [14]. This discrepancy may indicate a difference in the inherent toxicities of some crustacean haemolymph to bacteria. It could also be due to procedural differences [15]. The present study indicatesthat haemolymph of *p. segnis* crab may contain potential antibiotics. The antimicrobial assay done so far will serve as a baseline data for further studies that may confirm the hypothesis that brachyuran crabs haemolymphare indeed potential sources of novel compounds with biological potential. The revealing and development of the antimicrobial compounds in the haemolymph will provide an opportunity for the production of new compounds with natural activities as alternatives to antibiotics. Further purification of the active compounds is necessary in order to identify their chemical nature and to evaluate their potency as novel drug.

Impact of study

In the present study antimicrobial compounds were isolated for the first time from the hepatopancreas and hemolymph of the blue swimming crab, Portunussegnis.

CONFLICT OF INTERESTS

Declared None

REFERENCE

1. Ravichandran, SS Wahidulla, L D Souza, G Rameshkumar. Antimicrobial lipids from the hemolymph of brachyuran crabs. J Appl Biochem Biotechnol 2010;162(4):1039-51.
2. Smith VJ, JR Chisholm. Antimicrobial protein in crustaceans. J Adv Exp Med Biol 2001;484:95-112.
3. Rameshkurmar, GS Ravichandran, G Kaliyavarathan, TT Ajithkumar. Antimicrobial peptide from the crab, *Thalamitacrenata* (Latreille, 1829). World J of Fish and Marine Sci 2009;1(2):74-9.
4. Stewart JE, BM Zwicker. Natural and induced bactericidal activities in the hemolymph of the lobster; Homarus americanus: products of hemocyte-plasma interactions. Canadian J of Microbiology 1972;18:1499-509.
5. Rameshkumar, GT Aravindhan, S Ravichandran. Antimicrobial proteins from the crab *Chrybdislucifera* (Fabricius, 1798). Middle-East J of Scientific Res 2009;4(1):40-3.
6. Miyata T, F Tokunaga, T Yoneya, K Yoshikawa, S Iwanaga, M Niwa, T Takao, Y. Shimonishi. Antimicrobial peptides, isolated from horseshoe crab hemocytes, Tachyplesinm II and PolyphemisinsI and II:chemical structures and biological activity. J of Biochemistry 1989;106:663-8.
7. Veeruraj, A S Ravichandran, G Rameshkumar. Antibacterial activity of crab hemolymph on clinical pathogens. J Trend in Applied Sci Res 2008;3:174-81.
8. Anitha A, K Sharath. Antimicrobial peptides from the haemolymph of crabs *Scylla serrata* and *Metapograpsusmessor*. Asiatic J of Biotechnology Resources 2(5):568-76.
9. Chodsang S, S Benjakul, W Visessanguan, K Hara, A Yoshida, X Liang. Low molecular weight trypsin from hepatopancreas of freshwater prawn(*Macrobrachiumrosenbergii*):characteristics and biochemical properties. J Food Chemistry 2012;134:351-8.
10. Baure AW, WMM Kirby, MJC Sherris, M Turck. Antibiotic susceptibility testing by a standardized single disc method. American J of Clinical Patbol 1996;45:493-49.
11. Edward N J, T A Arroll, Z Fan. Specificity some physiochemical characteristics of antibacterial activity from blue crabs*Callinectessapidus*. J Fish Shellfish Immunol 1996;6:403-12.
12. Hoq M I, M U Seraj, S Chowdhury. Isolation and characterization of antibacterial peptides from the mud crab *Scylla serrata*. Pakisthan J of Biological Sci 2003;6(11):1345-53.
13. Schnapp D, GD Kemp, VJ Smith. Purification and characterization of a prolin rich antibacterial peptide, with sequence similarity to bactenecin-7, from the hemocytes of the shore crab, *Carcinusmaenas*. European J of Biochemistry 1996;240:532-39.
14. Anbuchezhian RM, S Ravichandran, GRameshkumar, T T Ajithkumar. Influence of crab haemolymph on clinical pathogens. Adv in Biological Res 2009;3(3-4):104-9.
15. Kumaravel K, Ravichandran S, Sritama BS. *In vitro* antimicrobial activity of shrimpshaemolymph on clinical pathogens. African J of Microbiology Res 2010;4(23):2592-6.
16. Nakamura T, Furanaka H, Miyata T, Muta F, Iwanaga S, Niw M, Takao T, Shimonishi, Y. 1988. Tachyplesin, a class of antimicrobial peptide from the hemocytes of the horseshore crab (*Tachypleustridentatus*). J Biol Chem 263:16709-13.
17. Gudmundsson CG, Lidholh DA, Asling B, Gan R, Boman HG. Thececropin locus:cloning and expression of a gene cluster encoding three antibacterial peptides in *Hyalophoracecropia*. J Biol Chem 1991;266:11510-17.
18. Chisholm JRS, Smith VJ. Antibacterial activity in the hemocytes of the shore crab, *Carcinusmaenas*. J Mar Biol Ass UK 1992;72:529-42.
19. Destoumieux D, Bulet P, Loew D, Dorsselaer V A, Rodriguea J, Bachere E. Penaedine, a new family or antimicrobial peptides isolated from the shrimp *penaeusvannamei*(Decapoda). J Biol Chem 1997;72:28398-406.
20. Ravichandran S, Jeyalakshmi S, Sudha S, Anbuchezhian R. Antimicrobial peptides from the haemolymph of the prawn *Macrobrachiumrosenbergii*. J of the Bangladesh Pharm Society (BDPS) 2010;5:6267.
21. Noga EJ, Arroll TW, Fan Z. Specificity and some physiochemical characteristics of the antibacterial activity from the blue crabs *Callinectessapidus*. Fish Shellfish Immunol 1996;6(6):403-12.
22. Cuthbertson BJ, Bullesbach EE, Gross PS. Discovery of synthetic penaeidin activity against antibiotic resistant. Chem Biol Drug Des 2006;68:120-7.
23. Kawababa SI, Nagayama R, Hirata M, Shigenaga T, Agarwala KL, Saito T, *et al*. Tachycitin, a small granular component in horseshore crab hemocytes is an antimicrobial protein with chitin-binding activity. J Biochem 1996;120:1253-60.
24. Ravichandran S, Rameshkumar G, Anthonysa y A, Kumaravel K. Antibacterial activity from the haemolymph of some Brachyuran crabs. American-Eurasian J of Scientific Res 2009;4(1):26-33.