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RESEARCH ARTICLE

COMPARATIVE ANALYSIS OF THE ANTMICROBIAL POTENTIAL OF HEMOLYMPH AND HEMOLYMPH LECTIN OF THE MARINE CRAB *GRAPSUS TENUICRUSTATUS*

Rathika R K.[1,*], Mary Mettilda Bai S.[1], Vinoliya Josephine Mary J.[1], Citarasu T.[2] and Vargila F.[1]

[1]Department of Zoology, Holy Cross College (Autonomous), Nagercoil- 629 004
[2]Centre for Marine Science and Technology, Manonmaniam Sundaranar University, Tirunelveli

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ABSTRACT

Marine crabs are potential sources of new antibiotics as the circulating hemolymph contains biologically active substances such as complements, lectins, clotting factors and antimicrobial peptides. The present study was focused on evaluation of antimicrobial potential of the hemolymph and the lectin isolated from the hemolymph of marine crab *Grapsus tenuicrustatus*. The antimicrobial activity of crude hemolymph, clarified serum and purified lectin of the experimental crab was tested by disc diffusion method. The hemolymph exhibited higher activity than clarified serum and purified lectin. Maximum activity of 14 mm was recorded against *Pseudomonas aeruginosa*. Antimicrobial activity was also observed against other tested bacteria such as *Bacillus subtilis, Staphylococcus aureus, Enterobacter aerogenes* and *E. coli*. Among the fungal strains tested maximum activity of 13 mm and 15 mm was recorded with hemolymph and clarified serum against *Aspergillus niger*. The results indicate the antimicrobial efficiency of the hemolymph and the lectin and hence these would be considered as good source of antimicrobial agents and would replace the existing inadequate and cost effective antibiotics.

Key words: Lectin, *Grapsus Tenuicrustatus*, Hemolymph, Antimicrobial, Gram Positive and Gram Negative.

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INTRODUCTION

Marine crabs are potential sources of new antibiotics as the circulating hemolymph in marine crustaceans contains biologically active substances such as complements, lectins, clotting factors and antimicrobial peptides (Veeruraj et al. 2008). Agglutinins or lectins are conventionally defined as glycoproteins of non-immune origin with a remarkable ability to interact with specific carbohydrate structures present on cell surfaces, extracellular matrices or secreted glycoproteins (Goldstein et al. 1980). Lectins defend against pathogenic bacteria and fungi by recognizing and immobilizing the infecting microorganisms via binding to carbohydrates on microbial surfaces, thereby preventing their subsequent growth and multiplication (Qadir et al. 2013) and considered as potent antimicrobials. Antimicrobial mechanisms of lectins include the pore formation ability, followed by changes in the cell permeability and latter, indicates interactions with the bacterial cell wall components. In addition, the antimicrobial activity of lectins is associated with the chitin-binding property, resulting in the disintegration of the cell wall or the arrest of de novo synthesis of the cell wall during development or division.

Corresponding author: **Rathika R K.,**
Department of Zoology, Holy Cross College (Autonomous), Nagercoil- 629 004.

Hence the present investigation was taken up to study the antimicrobial activity of the hemolymph and lectin isolated from the hemolymph of an interesting brachyuran crabs, the sally light foot crab or natal sally light foot or shore crab, *Grapsus tenuicrustatus* against few bacterial and fungal strains.

MATERIALS AND METHODS

Animal Collection: The marine crab *Grapsus tenuicrustatus* were collected from Kadiyapatanam (8.1262°N latitude and 77.3196°E longitude) and Muttom (37.6428°N latitude and 78.3924°E longitude) coasts, Kanyakumari, Tamilnadu, India.

Sample Preparation: Hemolymph, serum and the hemolymph lectin were the samples used for this study. Hemolymph from the crab was collected and then the serum was prepared (Mercy and Ravindranath, 1993). The presence of agglutinin/ lectin in the hemolymph was identified by hemagglutination (HA) and hemagglutination inhibition (HAI) assays (Ravindranath and Paulson 1987, Mercy and Ravindranath 1993). The hemolymph lectin was purified by using biospecific adsorption method of Nowak and Bamodes (1975) with slight modifications (Mary Mettilda Bai and Basil Rose, 2020).

Experimental Microbes: Five pathogenic bacterial strains both Gram positive bacteria (*Bacillus subtilis* (MTCC 5981), *Staphylococcus aureus* (MTCC 737)) and Gram negative bacteria (*Pseudomonas aeruginosa* (MTCC 424), *Enterobacter aerogenes* (MTCC 111), *Escherichia coli* (MTCC 443) and two fungal strains *Pencillium chrysogenum* (MTCC 5108), *Aspergillus niger* (MTCC 282) were obtained from Smykon Biotech, Nagercoil.

Antibacterial Assay: *In vitro* antibacterial assay was carried out by disc diffusion technique (Bauer et al. 1996). Whatmann No. 1 filter paper disc with 6 mm diameter was impregnated with 20 µl of the hemolymph, clarified serum and purified hemolymph lectin. The impregnated discs along with positive control (standard antibiotic disc) were kept on the Muller Hinton Agar (MHA) plates seeded with test bacterial cultures (*Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Enterobacter aerogenes* and *E. coli*). After incubation at room temperature for 24 hour, antibacterial activity expressed in terms of diameter (mm) of zone of inhibition was measured and recorded.

Antifungal Assay: *In vitro* antifungal activity was determined using the technique of Bauer et al. (1996). Two different species of fungal pathogen (*Pencilium chrysogenum* and *Aspergillus niger*) were inoculated by spread plate method using 0.1 ml of 72 hour old culture, maintained in Potato Dextrose Broth (PDB). Whatmann No.1 filter paper (6 mm) discs impregnated with 20 µl of hemolymph, clarified serum and purified hemolymph lectin and positive control with a standard antibiotic disc were placed on the Potato Dextrose Agar (PDA) plates seeded with test fungal cultures. After incubation at 30°C for 24 hours, antifungal activity was measured in terms of zone of inhibition (Anbuchezhian et al. 2009).

RESULTS

Antibacterial Activity: The zone of inhibition of bacterial strains caused by hemolymph, clarified serum and purified lectin is shown in the table 1. The hemolymph showed better effect than the clarified serum and purified lectin on bacterial strains. Among the tested samples maximum activity (14 mm) was recorded against *Pseudomonas aeruginosa,* minimum against *Enterobacter aerogenes* (8 mm) and *E. coli* (Figure 1).

Antifungal Activity: Fungal strains *Pencillium chrysogenum* and *Aspergillus niger* were used to test the antifungal activity of the lectin. Maximum activity of 13 mm was recorded with hemolymph and minimum activity was recorded with purified lectin against *Pencillium chryosgenum.* Maximum activity (15 mm) was observed with clarified sreum and minimum activity with purified lectin (Table 2, Figure 2) against *Aspergillus niger.*

Table 1: Antibacterial activity of the hemolymph, serum, hemolymph lectin of the crab, *G. tenuicrustatus*

Sample	Zone of inhibition (mm)				
	Bacteria				
	Gram positive		Gram negative		
	Bacillus subtilis	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Enterobacter aerogenes*	*Escherichia coli*
Hemolymph	13	10	14	11	11
Clarified serum	10	9	10	8	9
Purified lectin	8	7	7	6	8
Standard	25	31	26	22	21

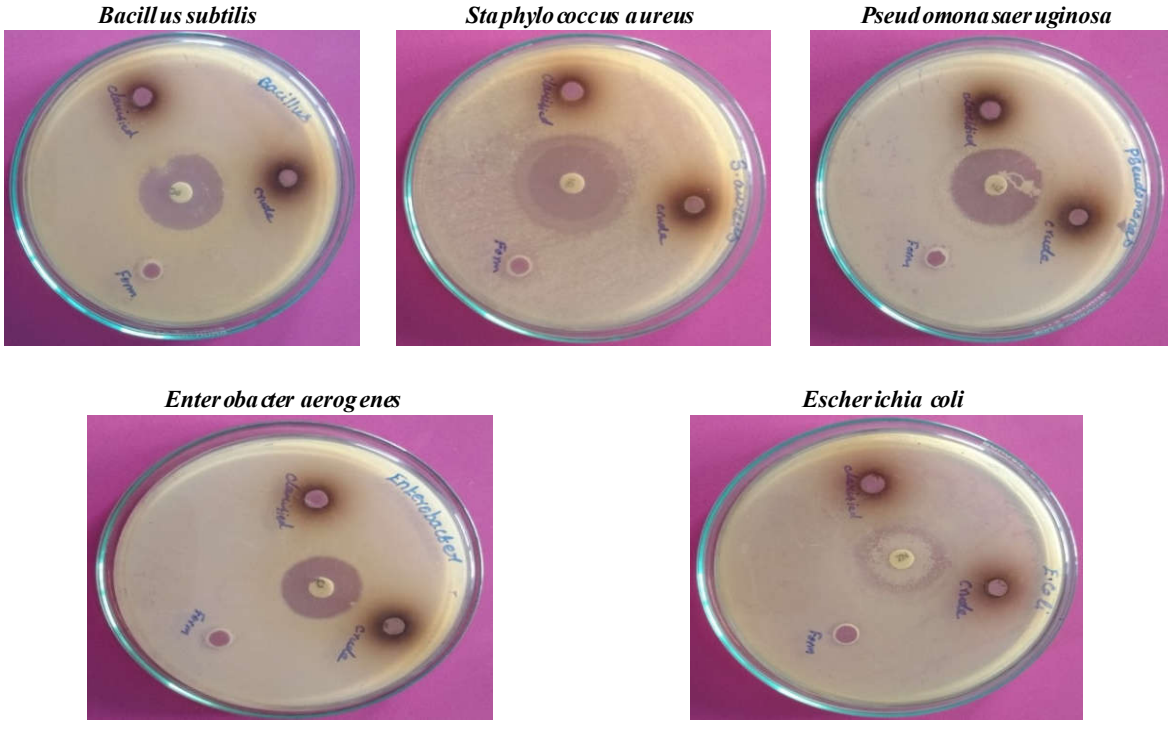
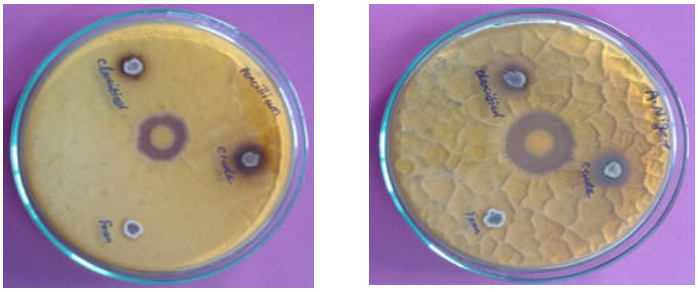


Figure 1: Antibacterial activity of hemolymph, serum and hemolymph lectin of the crab *G. tenuicrustatus*

Table 2: Antifungal activity of the hemolymph, serum, hemolymph lectin of the crab, *G. tenuicrustatus*

Sample	Zone of inhibition	
	Fungus	
	Pencillium chrysogenum	*Aspergillus niger*
Hemolymph	13	12
Clarified serum	9	15
Purified lectin	7	7
Standard	18	23



Pencillium chrysogenum **Aspergillus niger**

Figure 2. Antifungal activity of hemolymph, serum and hemolymph lectin of the crab *G. tenuicrustatus*

DISCUSSION

Agglutinins or lectins, ubiquitous carbohydrate binding proteins of humoral defense system are highly variable in their amino acid sequence and with different functions, structures, tissue localization and carbohydrate binding specificities and are involved in various biological functions, such as, host defense, cell-cell interaction and folding of glycoproteins. They are natural proteins with potent antimicrobial efficacy as they can bind specifically to the carbohydrates on microbial surfaces. Antimicrobial activities of hemolymph proteins (Cuthbertson et al. 2006; Kawababa et al. 1996) and hemolymph lectins (Krishnamoorthi, 2016) have been reported. Hence an evaluation of the antimicrobial activity of hemolymph and hemolymph lectin was made in this investigation.

The tested samples showed antimicrobial activity against both bacterial and fungal strains. Maximum activity was observed with hemolymph against *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Enterobacter aerogenes* and *E. coli* when compared to clarified serum and the purified lectin. Similar results were also observed with the fungal strains. The possible reason could be that apart from the lectin, various other factors like antimicrobial peptides, clotting factors or complements in the hemolymph would have been responsible for the antimicrobial activity. The antimicrobial activity of the hemolymph and serum might be due to constituents of innate immune system (Anbuchezhian et al. 2009). Decapods haemocytes are known to contain several immune effectors and they play a major role in the cellular and humoral defense mechanisms of the host (Ravichandran et al. 2010). As the hemolymph showed antimicrobial activity it could be confirmed that the haemocytes might be the site of production and storage for these antimicrobial peptides. It is believed that circulating haemocytes are playing an important role in the innate immune response of invertebrates, including being the storage reservoir of several immune components, such as lectins, coagulation factors and protease inhibitors (Hoq et al. 2003).

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 (Noga et al. 1994). The lectin purified from the hemolymph of the crab *G. tenuicrustatus* showed inhibitory activity to fungal and bacterial pathogens. The concentration of protein in the hemolymph shows wide interspecific variation among the brachyuran crabs. The ability of the serum of *G. tenuicrustatus* to agglutinate the bacteria, particularly the potential pathogens implicates the possible involvement of the humoral agglutinins in host defense response as lectin is one of the antimicrobial compounds. Lectins are highly specific to microbes and the reason for moderate activity may be due to the quantity of lectin used for testing antimicrobial activity. If the dose of lectin is increased there may be a possibility for the increase in antimicrobial activity. The antimicrobial assay done so far will serve as a baseline data for further studies that may confirm the hypothesis that brachyuran crabs haemolymph and hemolymph lectin are indeed potential sources of novel compounds with biological potential. It is therefore concluded that hemolymph of the marine crab *G. tenuicrustatus* exhibits a wonderful resource of antimicrobial proteins, which can be used to avert the colonization of the microbes.

REFERENCES

Anbuchezhian, RM., Ravichandran, S., Rameshkumar, G & Ajithkumar, TT, 2009. 'Influence of crab haemolymph on clinical pathogens', Advances in Biological Resolution, vol. 3, no. 3-4, pp. 104-109.

Bauer, AW., Kirby, WM., Sherris, JC & Turck, M, 1996.'Antibiotic susceptibility testing by a standardized single disc method', American Journal of Clinical Pathology, vol. 45, no. 4, pp 493-496.

Cuthbertson, BJ., Bullesbach, EE & Gross, PS, 2006.'Discovery of synthetic penaeidin activity against antibiotic-resistant fungi', Chemical Biology & Drug Design, vol. 68, pp. 120-127.

Goldstein. I.J., Hughes. R. C., Monsigny. M., Osawa.T., and N. Sharon. (1980). What should be called a lectins?, Nature pp. 285 -296.

Hoq, MI., Seraj, MU & Chowdhury, S, 2003. 'Isolation and characterization of antibacterial peptides from the mud crab Scylla serrata', Pakisthan Journal of Biological Science, vol. 6, no. 11, pp. 1345-1353.

Kawababa, SI., Nagayama, R., Hirata, M., Shigenaga, T., Agarwala, KL & Saito, T 1996. 'Tachycitin, a small granular component in horseshore crab hemocytes is an antimicrobial protein with chitin-binding activity', Journal of Biochemistry, vol. 120, pp. 1253-1260.

Krishnamoorthi, A., Selvakumar, S & Shanthi, G, 2016. 'Antimicrobial activity of lectins isolated from the marine crab *Scylla serrata*', International journal of Modern Research and Reviews, vol.4, no.11, pp. 1350-1352.

Mary Mettilda Bai, S & Basil Rose, MR, 2020. 'Purification and characterization of hemolymph lectin of freshwater crab, *Lamella lamellifrons* (Alcock, 1909)', Annals of Tropical Medicine & Public Health vol. 23, no. 7, pp. 1174-1187.

Mercy, PD & Ravindranath, MH, 1993. 'Purification and characterization of N-glycolyl neuraminic acid – specific lectin from *Scylla serrata*,' European Journal of Biochemistry, vol. 215 No. 3 pp. 697-704.

Nowak, TP & Barondes, SH, 1975. 'Agglutinin from *Limulus polyphemus*: Purification with formalinized horse

erythrocytesas the affinity adsorbent', Biochemica Biophysica Acta, vol. 393, pp.115-123.

Noga, EJ., Engel, DP., Arroll, TW., Mckenna, S & Davidian, M, 1994. 'Low serum antibacterial activity coincides with increased disease in blue crabs *Callinectussapidus*', Diseases of Aquatic Organisms, vol. 19, pp. 121-128.

Qadir, S., Hussain, IW.,Rafiq, S., Ahmad, GS & Masood, 2013. 'A Evaluation of antimicrobial activity of a lectin isolated and purified from *Indigo feraheterantha* Rabia Hamid', Advances in Bioscience and Biotechnology, vol. 4, pp. 999-1006.

Ravindranath, MH & Paulson, JC, 1987. 'O-acetyl sialic acid specific lectin from the crab *Cancer antennarius*', Methods ofEnzymology, vol.138, pp.520-527.

Ravichandran, S., Jeyalakshmi, S., Sudha, S & Anbuchezhian, R, 2010. 'Antimicrobial peptides from the haemolymph of the prawn *Macrobrachiumrosenbergii*', Journal of the Bangladesh Pharm Society (BDPS), vol. 5, pp. 6267.

Veeruraj, AS., Ravichandran, G & Rameshkumar, 2008. 'Antibacterial activity of crab hemolymph on clinical pathogens', Journal ofTrends in Applied Science Res, vol. 3, pp. 174-181.

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