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Antimicrobial Potential of Hemolymph of A Fresh Water Crab *Oziotelphusa Senex Senex* ((Fabricius 1798)

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Abstract: Crustaceans, have an immense immunological defence against pathogenic microorganisms. In the present study, effort has been made to find the antimicrobial activity of haemolymph collected from a freshwater crab *Oziotelphusa senex senex*. The hemolymph collected was subjected to antimicrobial assay by well diffusion method against clinical pathogens. Six bacterial species namely *Escherichia coli, Klebsiella pneumonia, Streptococcus pyrogenes Pseudomonas aeruginosa, Staphylococcus aureus and Bacillus sp.* The result shows a strong response of haemolymph against the clinical pathogens which confirms the immune mechanism of the freshwater crab.
Keywords: *O senex senex*, AMP, Hemolymph, antibacterial activity, pathogens.

Introduction

The emergence of new infectious diseases and resistance to antibiotics by the existing ones led to the development of new drug discovery(1).Antimicrobial peptides are important in the first line of the host defense system of many animal species. Their value in innate immunity lies in their ability to function without either high specificity or memory. Moreover their small size makes easy to synthesize without dedicated cells or tissues and they rapidly diffuse to point of infection(2). Recent experimental data from invertebrates suggests the past exposure to pathogens in individual animals can lead to enhanced immunity and some are also known to have considerable specificity by recognizing non self pathogen associated receptors that are highly conserved in evolution(3). Some of the known innate responses in invertebrates include phagocytosis, nodulation and encapsulation, synthesis of AMP and activation of proteolytic cascades that lead to melanization, blood coagulation, release of stress responsive proteins and molecules believed to function in opsonization and iron sequestration(4). The crabs are in intimate contact with aquatic environment rich in pathogenic microbes and are prone to infection by those microbes at various stages of growth, and losses due to disease can be enormous (5). An. Over the past several years, many antimicrobial peptides have been found and characterized in crab species. The first antimicrobial pepitde characterised was a proline peptide of 6.5KDa from the hemocytes of the shore crab Carinus maenas (6). The antimicrobial peptide Callinecin is a cationic antimicrobial peptide of 3.7 KDa isolated from the blue crab, *Callinectus sapidus*. (7).Recently, scygonadin, an anionic antimicrobial peptide isolated from seminal plasma of the mud crab *Scylla serrata*(8).

Antimicrobial activity has been detected in several decapod crustaceans, including lobsters, crabs, shrimps and freshwater crayfish (9,10). Fresh water crabs are an important of the fauna of limnic environments (11).About 1300 species of fresh water crabs are distributed throughout the tropics and subtropics(12).Some of the Brachyuran crabs have shown pronounced activities and may be useful in the Biomedical area .The

potential of Freshwater crabs as a source of biologically active products is largely unexplored. The circulating hemolymph in crustaceans contain biologically active substances such as complement, lectins, clotting factors and antimicrobial peptides [13].Hence a broad screening of Freshwater crabs for bioactive compounds is necessary.The present study was aimed to analyze antibacterial activity of hemolymph from *Oziotelphusa senex senex.*

Sample collection

Freshwater Crab (*Oziotelphusa senex senex)* was collected from the paddy field in Kundratur, Thiruvallur district, Tamilnadu, India..Healthy Male and female crabs of uniform size and free from disease were used for experimental purpose and each crab was subjected to single bleed collection . The weight of the collected crabs was ranging between 50-100g. The crabs were acclimatized for a week in the laboratory.

Collection of hemolymph

Haemolymph of *O. senex senex* was collected aseptically from the base of one of the second walking legs using a sterile syringe. To avoid haemocyte degranulation and coagulation, the hemolymph was collected along with ice-cold citrate EDTA buffer (510m M NaCl ; 0.1M glucose; 30mM trisodium citrate; 20mM citric acid; 10mM EDTA, pH 4.6)(14) as anticoagulant. Haemolymph was centrifuged at 2000rpm for 15min at 4°C.Supernatant was collected by aspiration and stored at 4°C until use.

Protein Estimation Assay

The protein content of crude haemolymph samples were estimated by Lowry's method using Bovine serum albumin as standard [15] using UV spectrophotometer.

Microbial strains used

Antibacterial activity of crab was determined against 6 different bacterial strains viz, *Klebsiella pneumonia ,Escherichia coli, Pseudomonas aeruginosa , Streptococcus pyogenes Bacillus subtilis , Staphylococcus aureus*

Preparation of inoculum

Strains of 6 bacterial cultures were used in this study. Identical colonies were isolated and sub cultured in 2 ml of nutrient broth for 4 hrs at 37^{o}C.

Antimicrobial activity

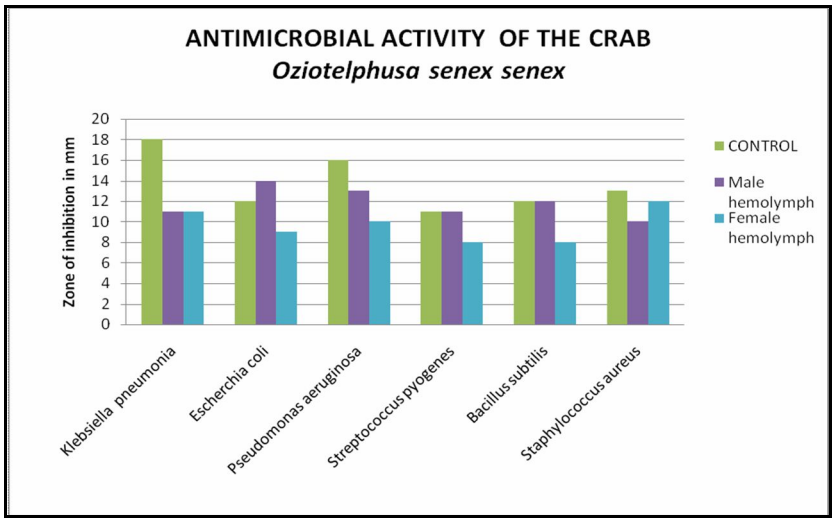
In vitro antibacterial assay was carried out by using standard well diffusion method (16,17]. 100 µL of diluted bacterial suspension (5 x 10^6 cfu /ml) of test bacterial strains were swabbed on the surface of Hinton agar. After 5 min, 100µl of hemolymph was added and the plates were incubated at 37^0C for 24 hrs. The diameters of the zone of inhibition of growth was measured with the help of a scale. More than 12 mm in the measurement is sensitive zones, between 4 to 12 mm is moderately sensitive and zones less then 4 mm are resistant.

Results

Antimicrobial Assay

Antibacterial Assay

Antibacterial activity of the male and female hemolymph sample of *O.senex senex* was used for the present study. The zone of inhibition in different bacterial strains against *O.senex senex* hemolymph is shown in (Fig 1). In antibacterial activity the highest zone of inhibition was observed in the hemolymph of male crab, *O.senex senex* against *E.coli* (14± 1mm) and the minimum activity was observed against *Staphylococcus aureus* (10 ± 1mm). In the hemolymph of female crab, *O.senex senex* the highest zone of inhibition was observed against *Klebsiella pneumonia* (11 ± 1 mm) and the minimum activity observed against *Streptococcus pyogenes and B acillus subtilis* (8 ± 1mm). The antibacterial agent of tetracycline showed activity against all the bacterial strains tested. The highest zone of inhibition was observed against *Klebsiella pneumonia* (18 ± 1mm) mm) the minimum activity observed against *Streptococcus pyogenes* (11 ± 1 mm).



S.NO	ORGANISM	Zone of inhibition in mm		
		Control	Male Haemolymph	Female Hemolymph
1	*Klebsiella pneumonia*	*18*	11	11
2	*Escherichia. Coli*	*12*	14	9
3	*Pseudomonas aeruginosa*	*16*	13	10
4	*Streptococcus pyogenes*	*11*	11	8
5	*Bacillus subtilis*	*12*	12	8
6	*Staphylococcus aureus*	*13*	10	12

Discussion

In recent years, great attention has been paid to study the bioactivity of natural products due to their potential pharmacological utilization. The present research investigation is made on the basis of in search of antimicrobial peptides from the hemolymph of *O.senex senex,* a fresh water crab collected from the rice field environment. In the present study the crab hemolymph shows antimicrobial activity against different range of bacterial strains of both gram positive and gram negative bacteria. In decapods crustaceans, it is known that environmental changes may affect the immune ability to susceptibility against pathogen infection. Previous works show that decapod crustaceans contain factors with antibacterial activity, particularly in the hemolymph or in the hemocytes. Antibacterial activity was reported in different body-parts of *Pagurus bernhardus* (Hermit crab), *Pandalus borealis* (Northern shrimp), *Hyas araneus* (Spider crab) and *Paralithodes camtschatica* (King crab) [18] . A similar result was observed with the hemolymph of some brachyuran crabs against clinical pathogens [19] Antibacterial activity has been previously described in a wide range of crustacean species . Antimicrobial peptides have been established as key players in animal defense systems. An antimicrobial peptide, which is isolated from a decapod crustacean (a crab, named *Thalamita crenata*), possess an immense antibacterial activity (20). One of such antimicrobial protein from the crab hemolymph(*Charybdis lucifera*) has been extensively studied against *E.coli* and *P. aeuroginosa* (21) The influence of crab hemolymph against wide range of clinical pathogens proves that crustaceans are very good source of antimicrobial potence (22). Antibacterial peptides can also be induced in epidermal cells in response to wounding or infection in the cuticles [23]. The whole process of synthesizing antibacterial proteins may take few minutes or hours after the changes, and these are secreted into the haemolymph of which some are lysozyme [24] and andropin [25]. These proteins show strong resistance to the microbial Growth.. The present study indicates that haemolymph of *O.senex senex* 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 haemolymph are 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.

Conclusion

The biological significance of the presence of the AMP in the crab hemolymph is still unclear. Considerable effort is being put into investigating the therapeutic potential of these peptides. The present study indicates that the haemolymph of crab would be a good source of antimicrobial agents and would replace, the existing in adequate and cost effective antibiotics. Following these in our present study, the crab haemolymph showed strong activity against the growth of selected microbes. The result suggests that the crab can produce antimicrobial substances instantly to combat microbial infection.

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