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Short Communication

ANTIBACTERIAL ACTIVITY FROM HAEMOLYMPH OF FRESHWATER CRAB OF GENUS *MAYDELLIATHELPHUSA* AGAINST RESPIRATORY TRACT PATHOGENS

SHURVEER SINGH[1], PRASHANT ARYA[2], SATYANAND BAHUGUNA[1*], JAGAT PRAKASH MEHTA[2], GAURAV BHATT[1], ASHISH KUMAR CHOWDHARY[1], VIVEK BAHUGUNA[1]

[1]Department of Zoology and Biotechnology, [2]Department of Botany and Microbiology, HNB Garhwal University, Srinagar Garhwal, Uttarakhand, India 246174
Email: profsnbahuguna@rediffmail.com

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ABSTRACT

Objective: To study the antibacterial activity using haemolymph from a freshwater crab species of genus *Maydelliathelphusa* against respiratory tract pathogens.

Methods: 3 ml of haemolymph was collected from the live animal with a fine sterile syringe and tested for antibacterial assay by the well diffusion method. Different quantities viz. 10µl, 20µl and 30µl of haemolymph were loaded in agar plates having bacterial strains and kept for incubation at 37 °C for 24 h to test the development of bacteria. Further, the efficacy of haemolymph against bacteria was compared with antibiotic erythromycin, used as positive control.

Results: The results reported the strong inhibitory action of the haemolymph against the pathogens in the present study. The lowest zone of inhibition was observed against *Staphylococcus aureus (S. aureus)* and *Pseudomonas aeruginosa (P. aeruginosa)* and the highest zone of inhibition was observed against *Streptococcus pneumoniae (S. pneumoniae)*. Erythromycin, as a positive control and Di-Methyl Sulfoxide (DMSO), as a negative control were used to check the sensitivity of pathogens. The minimum inhibition concentration (MIC) of the haemolymph for the studied bacteria was ranged between 6.25 mg/ml and 12.5 mg/ml. Maximum inhibition % (151.12) and minimum inhibition % (12.87) of haemolymph was calculated for *S. pneumoniae* and *K. pneumoniae* respectively during the study.

Conclusion: Study concluded that the haemolymph has a good antibacterial activity as it suppresses the growth of the bacteria completely at very low concentrations.

Keywords: Freshwater Crab, Haemolymph, Antibacterial activity, Respiratory tract pathogens.

Crustaceans are the most numerous, diverse and prevalent animals on earth along with insects. They lack complex and highly specific adaptive immune system of vertebrates, which is based on lymphocytes, immunoglobulins and immunological memory. The innate immune system of crustaceans is primarily related to their blood or haemolymph and is comprised of cellular and humoral responses [1]. Crabs are used for the medicinal purpose all over the world since long time ago. Their use as human food was reported by [2], and their economic and medical importance is well documented by [3]. Living in an aquatic environment rich in microorganisms, crustaceans have developed an effective system for detecting and eliminating noxious microorganisms. The crabs are in intimate contact with an 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 [4].

They are the rich sources of bioactive compounds; researchers carried out the pharmacological properties of marine crabs [5], but not in freshwater crabs. Few studies were conducted on the bioactivity of crustaceans by [6-8]. There are so many researches on marine as well as freshwater organisms still they need to be screened further for the discovery of new and useful antibiotics. Hence, an effort has been made to study the antibacterial activity of the crab's haemolymph against some selected respiratory tract pathogens such as *S. pyogenes*, *S. pneumoniae*, *S. aureus*, *K. pneumoniae* and *P. aeruginosa* and compared its activity with standard antibiotic erythromycin.

Crab samples were collected from a local stream of district Udham Singh Nagar in Uttarakhand. Approx. 3 ml of haemolymph was collected from joints of walking legs of the animal with a fine sterile syringe. To avoid haemocyte degranulation and coagulation, the haemolymph was collected in the presence of sodium citrate buffer, pH 4.6 (2:1, V/V). An equal volume of physiological saline (0.85% NaCl, w/v) was added to it. To remove haemocytes from plasma, the haemolymph was centrifuged at 2000g for 15 min at 4 °C. The supernatant was collected and stored at 4 °C until use. The antibacterial activity of haemolymph was determined by agar well diffusion method [9]. 0.1 ml of 12-16 h incubated cultures of bacterial species were mixed in molten Mueller Hinton Agar medium and poured in pre-sterilized Petri plates. A cork borer (6 mm diameter) used to punch walls in solidified medium and loaded with different quantities of haemolymph such as 10µl, 20µl and 30µl respectively. DMSO was used as negative control and erythromycin as a positive control. The plates were incubated at 37 °C for the 24 h in Biological Oxygen Demand (BOD) incubator and the diameters of the zones of inhibition were measured in millimetre. Each sample was assayed in triplicate and the mean±SD values were observed.

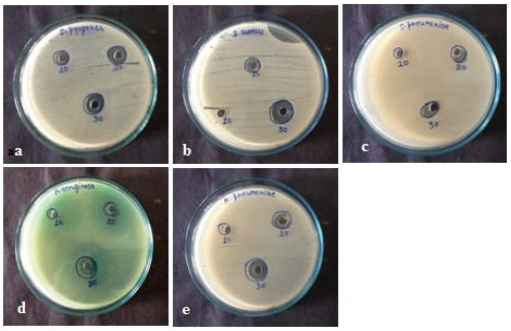

Fig. 1: Antibacterial activity against different bacterial strains at 10µl, 20µl and 30µl of haemolymph

Haemolymph of crab showed a significant antibacterial activity against the selected bacterial strains. The activity of haemolymph against *S. aureus* and *P. aeruginosa* was found minimum with a zone of inhibition 0.00 mm at 10µl (fig. 1b & d) whereas it was found maximum against *S. pneumoniae* with a zone of inhibition 10.66±1.2 mm at 30µl (fig. 1e).

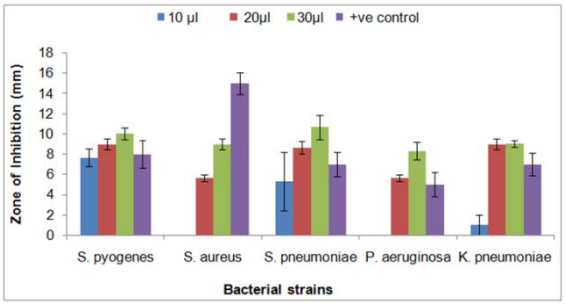

Fig. 2: Zone of Inhibition against different bacterial strains at 10µl, 20µl and 30µl of haemolymph and±ve control at 30µl, data presented as mean±SD of three replicates

The presence of antimicrobial compounds in the haemolymph of crustacean species (crabs) has been reported by so many researchers [10, 11]. Crab's haemolymph is known to contain several immune effects, and they play a major role in the innate immune mechanisms. The least activity of haemolymph was found against *S. aureus* and *P. aeruginosa* and maximum activity was against *S. pneumoniae.* Similar results were observed with the haemolymph of some brachyuran crabs against clinical pathogens [12]. In the present study, the haemolymph activity was more or less same against all the bacteria except *S. aureus,* where erythromycin gave a zone of inhibition (15±1.2 mm) that was almost double the haemolymph zone of inhibition (9±1.2 mm) fig. 2. This means *S. aureus* is more susceptible to erythromycin in comparison to others. The MIC of the haemolymph was ranged between 6.25 mg/ml and 12.5 mg/ml. The least MIC 6.25 mg/ml was obtained for *K. pneumoniae* among all the five bacteria. *S. pneumoniae* has maximum inhibition % (151.12) and *K. pneumoniae* has minimum inhibition % (12.87) against haemolymph during the study. The present findings suggest that this freshwater crab species are having a good antibacterial activity against bacteria causing respiratory tract infection in human beings and may be used effectively to treat the disease caused by them and would provide a new natural compound for the development of alternate antibiotics on further research.

CONFLICT OF INTERESTS

The authors declared no conflict of interest. Author Shurveer Singh is thankful to UGC New Delhi for providing Rajiv Gandhi National Fellowship (F.1-17/2011-12/RGNF-SC-UTT-6394) for conducting the present research work.

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