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Antibacterial activity from the hemolymph of freshwater crab, *Maydelliathelphusa masoniana* on some of clinical pathogens

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Abstract
In the present study, attempt has been made to assess the antimicrobial activity of haemolymph collected from a freshwater crab *Maydelliathelphusa masoniana*. The hemolymph collected was subjected to antimicrobial activities against clinical pathogens. Six bacterial species namely *Escherichia coli, Streptococcus pyrogenes Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis. and Salmonella paratyphi*. The antibacterial response of hemolymph found to vary with respect to sex. In male, the highest zone of inhibition was observed against *E.coli* while lowest in case of *Bacillus subtilis* whereas in female, the highest zone of inhibition was observed against *Streptococcus pyogenes* and the minimum in case of *Bacillus subtilis*.

Keywords: crab, hemolymph, antibacterial

Introduction
Different organisms have been used for medicinal purposes in various countries including India (Ravichandran *et al*., 2010) [2]. Due to emergence of antibiotic resistance bacterial strains several research has been carried out to find novel drugs. (Veeruraj *et al.*, 2008) [3] In crustacean, though the first line of defense is constitute by cuticle, however, once pathogen cross it, a complex interaction of innate humoral and cellular immune reaction is induced in both the tissues and haemocoel thereby helping to eliminate the pathogen at rapid pace (Ravichandran *et al*., 2010) [2]. As most of work on hemolyph as antibacterial has been confine to marine crabs, therefore, presently an attempt has been made to study the antibacterial activity of hemolymph in *M. masoniana* in some strain of bacteria. Our study will set a venture for future research on freshwater crabs as potential candidate for novel drugs with specific actions.

Materials and method
Sample collection
Freshwater crabs (*M. masoniana)* were collected from Gho-manhasan stream Jammu and brought to the laboratory. Healthy male and female crabs having 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 40-90g. The crabs were acclimatized for a week in the laboratory.

Collection of hemolymph
Haemolymph was collected aseptically from the base of one of the second walking legs using a sterile syringe. 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) as anticoagulant so as to avoid degranulation and coagulation. Haemolymph was centrifuged at 2000rpm for 15min at 4 °C. Supernatant was collected by aspiration and stored at 4 °C until use.

Microbial strains used
Antibacterial activity of crab was determined against 6 different bacterial strains viz, *Escherichia coli, Pseudomonas aeruginosa, Streptococcus pyogenes Bacillus subtilis, Staphylococcus aureus* and *Salmonella paratyphi*. The samples were obtained from Deptt. of Microbiology, University of Jammu.

Antimicrobial activity
In vitro antibacterial activites was determined by using standard technique described by Bauer *et al*., (5) 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 than 4 mm are resistant (Sumalatha *et al*., 2016) [1].

Results
Antimicrobial Assay
The present study was carried out to assess the antibacterial activity of hemolymph extracted from *M. masoniana*. Antibacterial activity of under study crab hemolymph was recorded to exhibit sex specific variations. The zone of inhibition in different bacterial strains against *M. masoniana* hemolymph is shown in (fig. 1). Out of the six bacterial strains (viz.,), zone of inhibition were observed to vary from 6.5mm, 7.0mm, 8.5mm, 9.5mm 12.5mm to 15mm against *Bacillus subtilis, Pseudomonas aeruginosa, , Staphylococcus aureus, Salmonella paratyphi, Streptococcus pyogenes* and *E.coli* respectively in male crabs of *M. masoniana*. Therefore, the highest zone of inhibition was observed in hemolymph of

male crab, *M. masoniana* against *E. coli* (15 mm) and the minimum activity was observed against *Bacillus subtilis* (6 mm). In the hemolymph of female crab, zone of inhibition recorded to be 7 mm (*Bacillus subtilis*), 9.5 mm (*Pseudomonas aeruginosa*), 10 mm (*Staphylococcus aureus*), 10 mm (*Salmonella paratyphi*), 10.5mm (*E.coli*) and 16.5mm (*Streptococcus pyogenes*), therefore, the highest zone of inhibition was observed against *Streptococcus pyogenes* and the minimum activity observed against *Bacillus subtilis*. The antibacterial agent of tetracycline showed activity against all the bacterial strains tested.

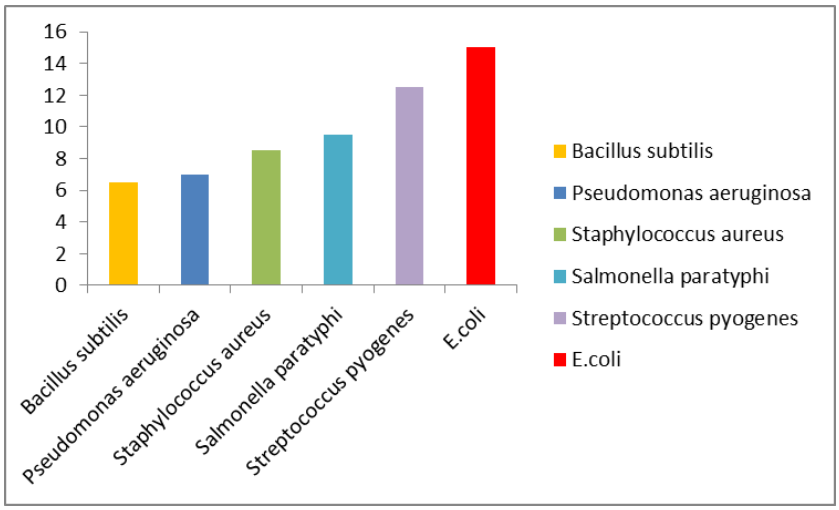

Fig 1: Zone of inhibition against hemolymph of male crab

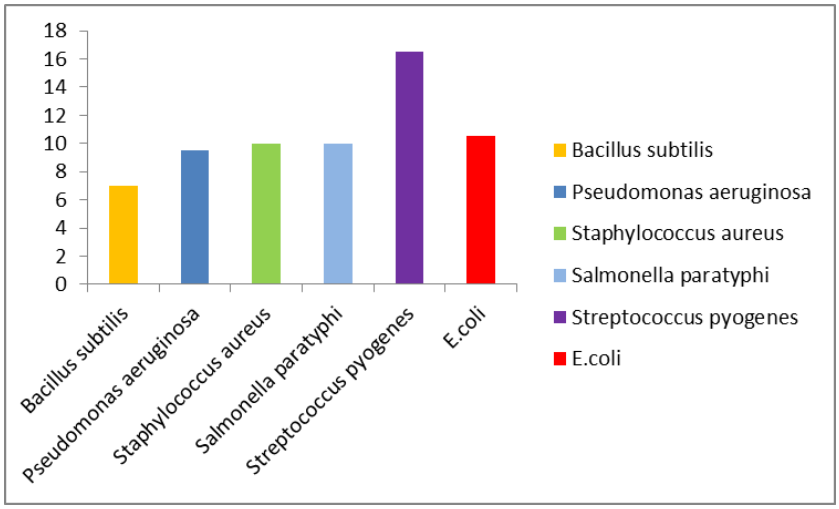

Fig 2: Zone of inhibition against haemolymph of female crab

Discussion

In the present study the crab hemolymph shows antimicrobial activity against different range of bacterial strains of both gram positive and gram negative bacteria. The antibacterial response of hemolymph recorded to vary in sex. In male, the highest zone of inhibition was observed against *E.coli* whereas least in case of *Bacillus subtilis* whereas in female the highest zone of inhibition was observed against *Streptococcus pyogenes* and the minimum activity observed against *Bacillus subtilis*. In similar tune, Sumalatha *et al*., 2016 [1] also held sex wise variations of hemolymph antibacterial activities, however in contrast to present study they reported the highest antibacterial activity of hemolymph in male was against *E. coli* while lowest in *Staphylococcus aureus* whereas in female highest and lowest activities recorded for *Klebsiella pneumonia* and *Streptococcus pyogenes* respectively. The variations of hemolymph response again bacteria might be attributed to species and environment difference as also held by Veeruraj *et al*., (2008) [3]. Previous works also show that decapod crustaceans contain factors with antibacterial activity, particularly in the hemolymph or in the hemocytes viz., Hyalinocytes, Semigranulocytes and Granulocytes (Gupta, 2013) [5]. Different workers had reported antibacterial activity in different body-parts of *Pagurus bernhardus* (Hermit crab), *Pandalus borealis* (Northern shrimp), *Hyas araneus* (Spider crab) and *Paralithodes camtschatica* (Mitta *et al*., 1999) [6].

As the hemolymph of crustaceans has potential to act against wide range of clinical pathogens thereby making them reliable candidate for very good source of antimicrobial potence. 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 (Lee and Brey, 1995) [7] and andropin (Samakovilis *et al*., 1991) [8]. These proteins show strong resistance to the microbial growth. The present study indicates that hemolymph of *M. masoniana* may contain potential antibiotics. The present attempt therefore will serve as a baseline data for further studies so as to confirm the hypothesis that brachyuran crabs haemolymph are indeed potential sources of novel compounds with biological potential.

Conclusion

Freshwater crabs like marine counterpart also have potency to produce antibiotics. Presently hemolymph of *M. masoniana* observed to produce zone of inhibition against some strain of bacteria, therefore intense research is needed to assess its antibacterial activity on other strains as well as on fungus. The research in this field will be helpful to generate a new source of antibiotic and might act effectively against present drug resistance strains.

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