Antibacterial activity from the haemolymph of freshwater crab, *Himalayapotamon emphysetum* on some of clinical pathogens

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Abstract

In the present study, an attempt has been made to assess the antimicrobial activity of haemolymph collected from a freshwater *Himalayapotamon emphysetum*. The haemolymph collected was subjected to antimicrobial activities against some of clinical pathogens viz., *Escherichia coli, Streptococcus pyrogenes Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis. and Salmonella paratyphi*. In males, the highest zone of inhibition was observed against *Staphylococcus aureus* while least in *Salmonella paratyphi* whereas in females, highest zone of inhibition was observed against *Streptococcus pyrogenes* while least in case of *Pseudomonas aeruginosa.*

Key words: Crab, Haemolymph, Antibacterial,

Introduction:

Invertebrates comprise over 95% of the animal species while some live in favourable and healthy environment whereas other face potentially harmful microorganism in their surroundings. As a result, these animals tend to develop various strategies so as to defend their live against harmful pathogens (Jiravanichpaisal *et al*., 2006). Freshwater crabs being in intimate contact with aquatic environment that may be rich in pathogenic microbes and thus always remain prone to infection (Hudson and Lester, 1994). Different organisms have been used for medicinal purposes in various countries including India (Ravichandran *et al*., 2010). Due to emergence of antibiotic resistance bacterial strains several research has been carried out to find novel drugs. (Veeruraj *et al.*, 2008) 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). 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 haemolymph in *M. masoniana* and *H. emphysetum* on 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* and *H. emphysetum*) were collected from Jhajjar stream, Jammu and brought to the laboratory. Healthy male and female crabs having uniform size as well as free from disease were used for experimental purpose and each crab was subjected to single bleed collection. The weight of the collected crabs was recorded to range between 40-90g. The crabs were acclimatized for a week in the laboratory.

Collection of haemolymph

Haemolymph was collected aseptically from the base of one of the second walking legs using a sterile syringe. The haemolymph 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*., (1996) 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).

Results

Antimicrobial assay from haemolymph of *H. emphysetum*

Out of six bacterial strains, zone of inhibition was observed to vary from 6 mm, 8.5 mm, 9 mm, 10.5 mm, 13 mm and 16.5 mm for *Salmonella paratyphi, Bacillus subtilis, Pseudomonas aeruginosa, Streptococcus pyogenes, E. coli* and *Staphylococcus aureus* in case of male whereas in female, the zone of inhibition recorded to be 9.5mm, 11.5mm, 7mm, 15.5 mm, 12 mm, 10.2mm for *Salmonella paratyphi, Bacillus subtilis, Pseudomonas aeruginosa, Streptococcus pyogenes, E. coli* and *Staphylococcus aureus* respectively.

Discussion

In the present study the crab haemolymph shows antimicrobial activity against different range of bacterial strains of both gram positive and gram negative bacteria. The antibacterial response of haemolymph further recorded to vary with sexes (figs. 1-2). Sex wise variations of haemolyph response against bacterial strain also has been reported by Gupta *et al*., (2017), however in contrast to present study, they held that in male crabs, *Maydelliathelphusa masoniana*, 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*, however, the minimum in case of *Bacillus subtilis*. The variations may be attributed to the fact that both the species inhabited different environmental conditions. In a similar tune, Veeruraj *et al*., (2008) while studying different species of crabs held that the antimicrobial activity of haemolymph changes with environmental conditions, thereby supporting the present point of view. Review of literature also have shown that decapods crustaceans contain factors with antibacterial activity may confine to haemolymph or in the hemocytes viz., Hyalinocytes, Semigranulocytes and Granulocytes (Gupta, 2012). 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).

As the haemolymph of presently studied crab species found to act against different strain of bacteria, the results therefore suggest that similar to *M. masoniana*, these crabs species can also produce antimicrobial substances instantly to combat bacterial infection. Crustaceans have potential to act against wide range of clinical pathogens thereby making them reliable candidate for very good source of antimicrobial potencies (Veeruraj *et al*., 2008). 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) and andropin (Samakovilis *et al*., 1991). These proteins show strong resistance to the microbial growth. 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: In conclusion, present study shows that the haemolymph of freshwater crabs, *H. emphysetum*, may contain several substances with antimicrobial activity. The determination of such component from haemolymph and their development as antimicrobial compounds in the haemolymph will provide an opportunity for the production of new compounds with natural activities as an alternative to antibiotics. Further purification of the active compounds is necessary in order to identify their chemical nature and to evaluate their potency as a novel drug.

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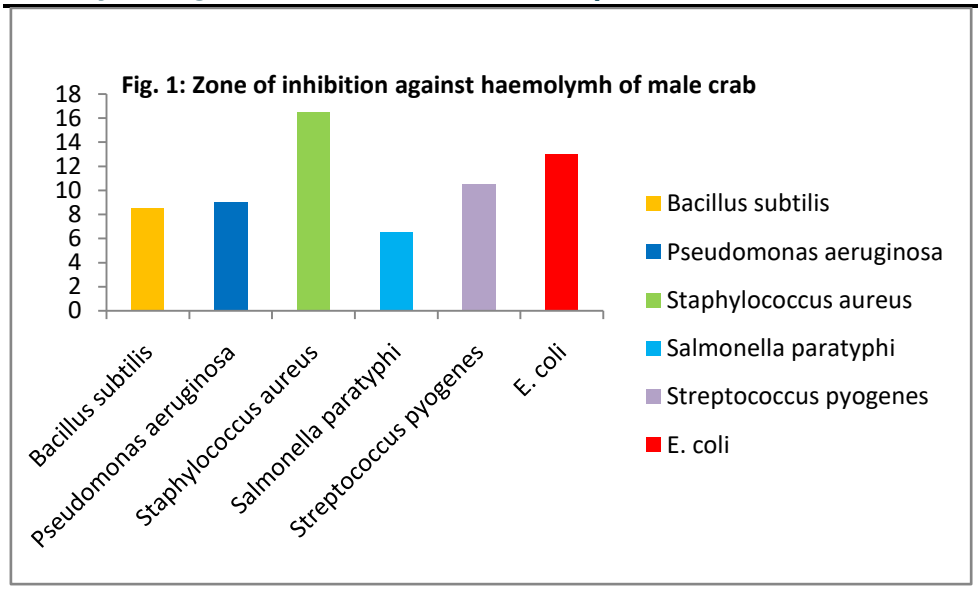
Fig. 1: Zone of inhibition against haemolymh of male crab
18
16
14
12
10
8
6
4
2
0
Bacillus subtilis
Pseudomonas aeruginosa
Staphylococcus aureus
Salmonella paratyphi
Streptococcus pyogenes
E. coli
Bacillus subtilis
Pseudomonas aeruginosa
Staphylococcus aureus
Salmonella paratyphi
Streptococcus pyogenes
E. coli

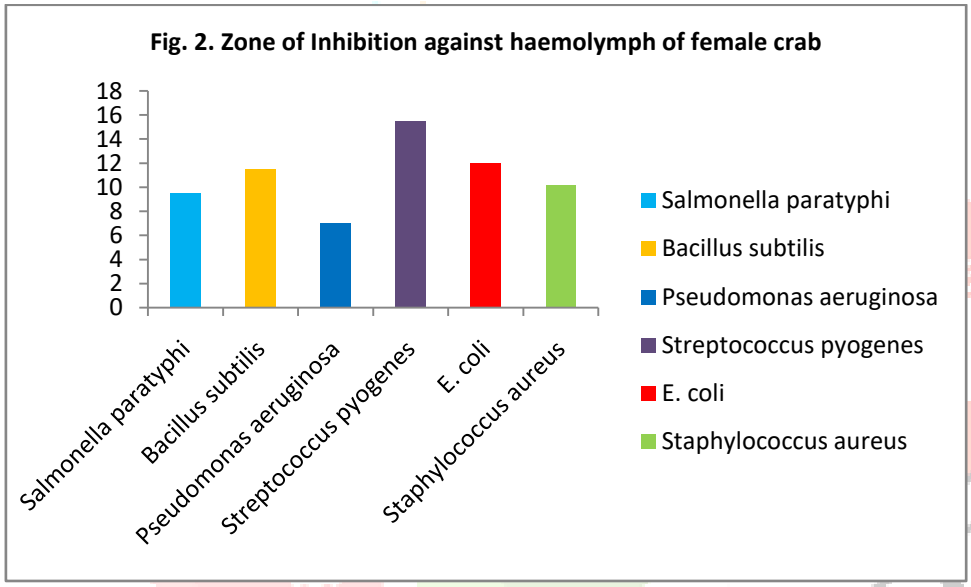
Fig. 2. Zone of Inhibition against haemolymph of female crab
18
16
14
12
10
8
6
4
2
0
Salmonella paratyphi
Bacillus subtilis
Pseudomonas aeruginosa
Streptococcus pyogenes
E. coli
Staphylococcus aureus
Salmonella paratyphi
Bacillus subtilis
Pseudomonas aeruginosa
Streptococcus pyogenes
E. coli
Staphylococcus aureus