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Antibacterial Activity of Crab Haemolymph on Clinical Pathogens

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Abstract: The present investigation was taken up to study the antibacterial activity of haemolymph extracts from six different species of crabs (*Scylla tranquebarica*, *S. serreta*, *Nanosesarma minutum*, *Neoepisesarma tetragonum*, *Metapograpsus maculatus and Macropthalmus depressus)*. Two positive controls Amphicillin (p1) and Erythromycin (p2) was also used. Investigation against a range of 10 different bacterial strains was used. The result demonstrated that the crab haemolymph of crude samples tested against gram positive and gram negative pathogenic bacterial strains and two antibiotic resistant strains were used. In antibacterial activity the highest zone of inhibition was observed in the haemolymph of *Scylla tranquberica* against *Vibrio cholerae* (10 mm) and lowest zone of inhibition was observed in the haemolyph of *M. depressus* against *S. paratyphi*-B and *S. typhi* (5 mm). The present study indicates that the haemolymph of crabs would be a good source of antimicrobial agents and would replace the existing inadequate and cost effective antibiotics.

Key words: Crabs, haemolymph, antibacterial, gram positive, gram negative

INTRODUCTION

Marine crabs are potential sources of new antibiotics. The search for antimicrobial agents has taken a definite direction in developed countries. The first line of defense of arthropods against pathogens and parasites is of physical nature via their hard cuticle. However, once this barrier is passed, a complex interaction of innate humoral and cellular immune reactions is induced in both tissues and haemocoel, which results in a fast elimination of micro-organisms (Bulet *et al*., 1999).

Review of literature on mangrove crabs shows most of the studies are on biodiversity, biochemistry and processing mangrove leaf litter (Lee, 1998; Ravichandran *et al*., 2000; Ravichandran and Kannupandi, 2004, 2005; Ravichandran *et al*., 2007a, b). But no proper studies on the antibacterial activity of crab haemolymph on clinical pathogens. Hence, a broad, based screening of marine crabs for bioactive compounds is necessary. A thorough understanding of biological activity will lead to the formulation of novel drugs with specific actions. The present investigation was taken up to study the antibacterial activity of crab haemolymph extracts from six different species of mangrove crabs (*Scylla tranquebarica*, *S. serreta*, *Nanosesarma minutum*, *Neoepisesarma tetragonum*, *Metapograpsus maculatus* and *Macropthalmus depressus*).

MATERIALS AND METHODS

Six species of crabs were collected from different sites of mangrove areas along the Vellar estuarine region (South east coast of India). Two species of mud crabs *Scylla serrata* and *S. tranquebarica* and another four semi terrestrial species of *N. minutum*, *N. tetragonum*, *M. maculatus* and *M. depressus* was collected by hand picking from mangrove and oyster bed region of Vellar estuary.

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Healthy male and female animals at different stages of development were used throughout for experimental purposes and each animal was subjected to a single bleed collections were being done at the time of use. Haemolymphs (approx. 3 mL) were collected by cutting each walking legs of the animal with a fine sterile scissor. To avoid haemocyte degranulation and coagulation, the haemolymph was collected in the presence of sodium citrate buffer, pH 4.6 (2:1, V/V). Equal volume of physiological saline (0.85% NaCl, w/v) was added to it. To remove haemocytes from plasma the haemolymph was centrifuged at 2000 g for 15 min at 4°C. Supernatant collected by aspirating and stored at 4°C until use.

Bacterial Strains Used

Antibacterial activity of mangrove crabs haemolymph was determined against 10 bacterial strains viz., *Lactobacillus bulgaris*, *Salmonella paratyphi*-B, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhi*, *Proteus mirabilis*, *Vibrio cholerae*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Klebsiella oxytoca*.

These pathogens strains were obtained from the department of medical microbiology (Raja Muthiyah Medical College and Hospital) Annamalai University, Annamalai nagar.

Anti Bacterial Assay

The spectrum of antibacterial activity was studied using as test agent a range of 10 different strains of human pathogenic gram positive and gram-negative bacteria of which there were two antibiotic agents (Amphicillin (P1) and Erythromycin (P2).

In vitro antibacterial assay was carried out by disc diffusion technique (Bauer *et al*., 1996) Whatman No. 1 filter paper discs with 4 mm diameter were impregnated with known amount test samples of the crabs haemolymph and positive control contained (250 mg) of a standard antibiotic disc. Negative controls not comprised sterile disc only. The impregnated discs along with control (incorporated with solvent alone) were kept at the center of Agar Plates, seeded with test bacterial cultures. After incubation at room temperature (37°C) for 24 h. Antibacterial activity was expressed in terms of diameter of zone of inhibition was measured in mm using caliper or a scale and recorded.

RESULTS

Biological Screening

Antibacterial activity of the haemolymph of six crabs showed a wide array of antibacterial activity. Highest zone of inhibition was observed in the haemolymph of *Scylla tranquberica* against *Vibrio cholerae* (10 mm) and lowest zone of inhibition was observed in the haemolyph of *M. depressus* against *S. paratyphi*-B and *S. typhi* (5 mm).

Antibacterial Activity

Antibacterial of *Scylla tranquberica*

The zone of inhibition in different bacterial strains against *S. tranqubarica* haemolymph is shown in Fig. 1-10. High measurement of zone of inhibition was recorded in *Vibrio cholerae* (10 mm) and lowest zone of inhibition was observed in *E. coli* strain (6 mm). Among the ten pathogenic strains *S. aureus*, *P. aeruginosa*, *P. mirabilis* and *K. oxytoca* alone shows negative activity and rest of them shown positive activity.

Antibacterial of *S. serrata*

The zone of inhibition varied from 6 mm to 6.5 mm. High diameter was noted against *Lactobacillus bulgaris* and all the other bacterial strains shows positive activity in 6 mm of zone of inhibition.

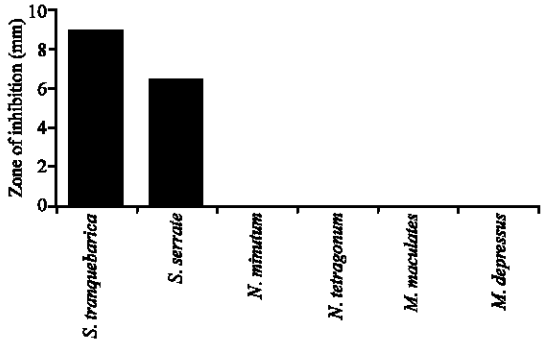

Fig. 1: Haemolymph of crabs against *L. bulgaris*

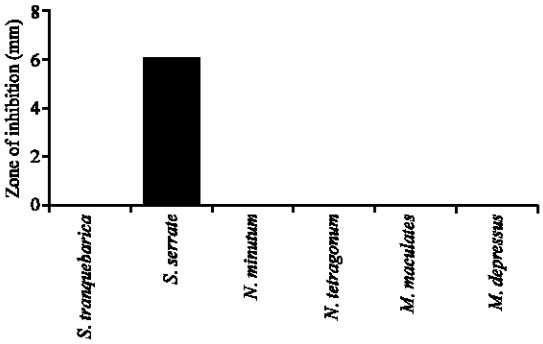

Fig. 2: Haemolymph of crabs against *S. aureus*

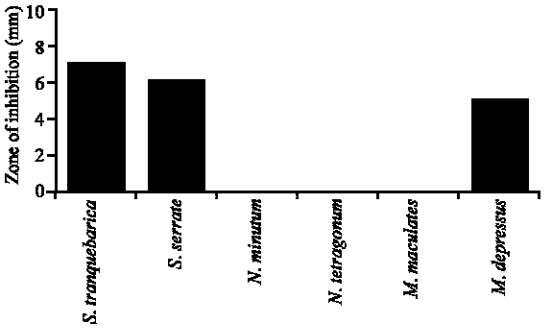

Fig. 3: Haemolymph of crabs against *S. paratyphi*-B

Antibacterial of *Nanosesarma minutum*

There was no antibacterial activity was recorded in the haemolymph of *N. minutum* against both gram positive and gram negative pathogenic strains.

Antibacterial of *Neoepisesarma tetragonum*

In the haemolymph of *N. tetragonum* also not showing any activity against the all pathogenic strains.

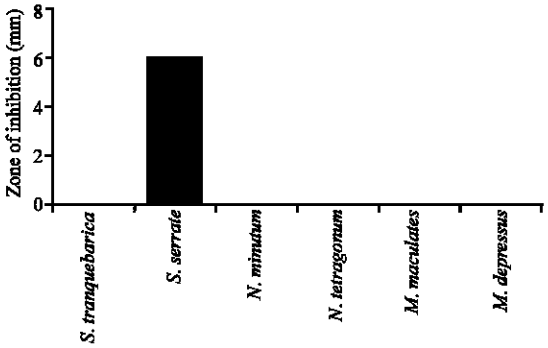

Fig. 4: Haemolymph of crabs against *P. aeruginosa*

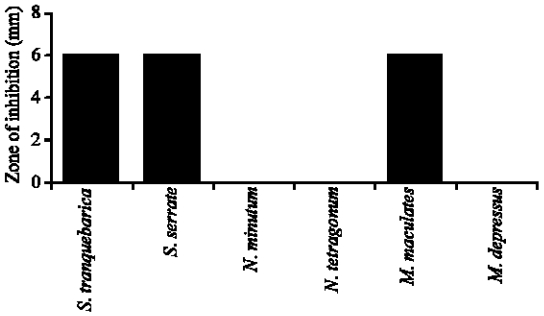

Fig. 5: Haemolymph of crabs against *E. coli*

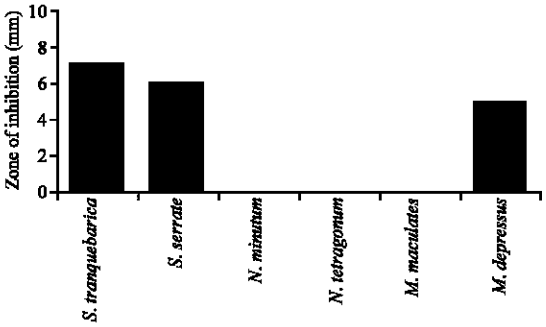

Fig. 6: Haemolymph of crabs against *S. typhi*

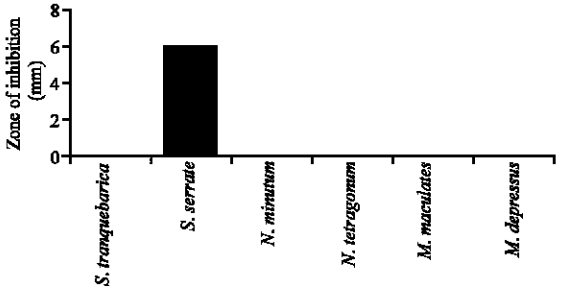

Fig. 7: Haemolymph of crabs against *P. mirabilis*

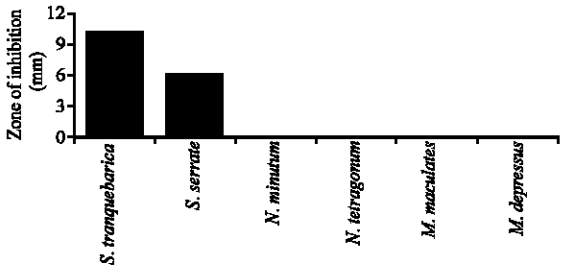

Fig. 8: Haemolymph of crabs against *V. cholerae*

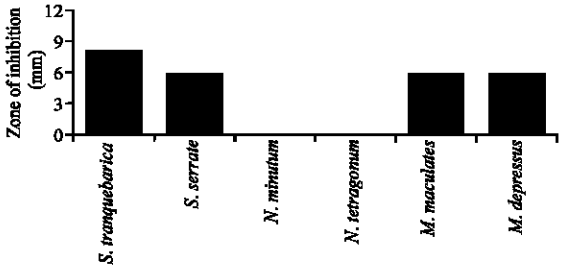

Fig. 9: Haemolymph of crabs against *K. pneumoniae*

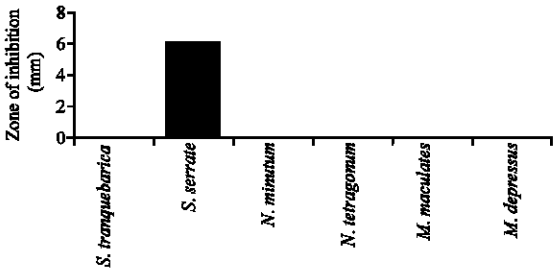

Fig. 10: Haemolymph of crabs against *K. oxytoca*

Antibacterial of *Metapograpsus maculatus*

The maximum inhibition zone (6 mm) was observed in *E. coli* and *K. pneumoniae*. But other strains like *L. bulgaris*, *S. aureus*, *S. paratyphi*-B, *P. aeruginosa*, *S. typhi*, *P. mirabilis*, *V. cholerae* and *K. oxytoca* are not showing any activity.

Antibacterial of *Macropthalmus depressus*

The maximum activity was showed against *K. pnemonia* (6 mm) and minimum zone was noted in *Salmonella paratyphi*-B and *S. typhi* (5 mm) and other pathogenic strains are not showing any activity.

Antibacterial Agents Amphicillin (P1) and Erythromycin (P2)

The extract of antibacterial agent of *Amphicillin* showed activity against all the bacterial strains tested. The maximum activity showed against *Lactobacillus bulgaris* (32 mm), The minimum activity observed against *Klebsiella pneumoniae* and *K. oxytoca* (7 mm). A similar trend of activity was

Table 1: Antibacterial activity of antibacterial agents from (Amphicillin (ρ1) and Erythromycin (ρ2))

Name of the Bacteria	Gram +Ve/-Ve	Activity of Agents P1	Activity of Agents P2	Zone of inhibition (mm in diameter) procured by induced antibacterial agents P1	Zone of inhibition (mm in diameter) procured by induced antibacterial agents P2
Lactobacillus bulgaris	+ve	+ve	+ve	32	20
Staphylococcus aureus	+ve	+ve	+ve	24	10
Salmonella paratyphi-B	-ve	+ve	+ve	16	8
Pseudomonas aeruginosa	-ve	+ve	+ve	18	8
Escherichia coli	-ve	+ve	-ve	10	-
Salmonella typhi	-ve	+ve	+ve	14	7
Proteus mirabilis	-ve	+ve	+ve	30	16
Vibrio cholerae	-ve	+ve	+ve	17	13
Klebsiella pneumoniae	-ve	+ve	+ve	7	9
Klebsiella oxytoca	-ve	+ve	+ve	7	10

observed antibacterial agent of *Erythromycin* showed activity against all bacterial strains tested. The maximum activity showed against *L. bulgaris* (20 mm) and minimum activity of against *S. typhi* and *K. oxytoca* (7 mm). There was no activity against *E. coli* strain (Table 1).

DISCUSSION

The presence of naturally occurring in the haemolymph of several crustaceans has been well known since the beginning of the 20th century (Cantacuzene, 1919). The decapods crustaceans, it is known that environmental changes may affect the immune ability to susceptibility against pathogen infection. In the present investigation, haemolymph were collected from six different crabs viz. *S. tranqguebarica*, *S. serrata*, *N. minutum*, *N. tetragonum*, *M. maculatus* and *M. depressus* were subjected to antibacterial and antifungal assay.

In arthropods, antimicrobial compounds were mainly studied in chelicerates (Horseshoe crabs) and insects. Their involvement in the defense reaction is quite different in these two groups. In horseshoe crabs, they are mainly synthesized in haemocytes (invertebrate blood cells) where they are stored after processing within their cytoplasmic granules (Iwanaga and Kawabata, 1998). They are believed to be released into haemolymph through regulated exocytosis upon microbial stimulation. The presence of antimicrobial compounds has been reported in crustacean species including the crabs *Carcinus maenas* (Schnapp *et al*., 1996) and *Callinectes sapidus* (Khoo *et al*., 1999) but, to date no data were available.

In the present study, the crab haemolymph showed antimicrobial activity against a range of different pathogenic strains of both gram positive and gram negative bacterial strains including few antibiotic resistant strains. The result suggests that crab can produce antimicrobial substances instantly to combat bacterial infection. Induction of antibacterial compounds was also observed in case of sarcotoxin I (Okada and Natori, 1985) and sapecin (Matsuyama and Natori, 1988) in *Sarcophagi peregrine*, moricin (Hara and Yamakawa, 1995), lebocin (Chowdhury *et al*., 1995) and ceropin-B (Taniai *et al*., 1995) in *Bombyx mori*. As the haemolymph showed antibacterial compounds were secreted in response to immunization. Similar observations were also found by Nakamura *et al*. (1988) in *Tachypleus tridentatus*, Morishima *et al*. (1992) in *Bombyx mori*, Gudmundsson *et al*. (1991) in *Hyalophora cecropia*.

As described for Limulus (Toh *et al*., 1991) and to some extent for mammalian antimicrobial peptides, some of the penaeidens stored in blood cells appear to be released into haemolymph upon stimulation. Actually microbial stimulation is known to trigger haemocyte degranulation as one of the most immediate haemocytic reactions in crustaceans (Smith and Soderhall, 1983; Jonansson *et al*., 2000) and in the freshwater crayfish *Pacifastacus leniusculus*, degranulation was shown to be associated with a rapid decrease in RNA and protein synthesis in granular cells.

The present study indicated that antibacterial activity the highest zone of inhibition was observed in the haemolymph of *Scylla tranqueberica* against *Vibrio cholerae* (10 mm) and lowest zone of inhibition was observed in the haemolyph of *M. depressus* against *S. paratyphi*-B and *S. typhi* (5 mm). In conclusion in the present study indicates that the haemolymph of crabs would be a good source of antimicrobial agents and would replace the existing inadequate and cost effective antibiotics.

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