Shibana C et al. International Journal of Recent Research Aspects ISSN: 2349-7688, Special Issue: Conscientious Computing Technologies, April 2018, pp. 723-726

Antibacterial Activity of Crab Portunus Pelagicus from Three Stations of Gulf Of Mannar Coast.

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Abstract- **Marine crabs are potential sources of new antibiotics. The search for antibacterial agents has taken a definite direction in developed countries. The present investigation was taken up to study the antibacterial activity of methanol extract of the crab Portunus pelagicus were collected from three stations. Different concentrations (1.25mg,2.5mg and 5mg) of methanol extract of the crab tissue tested against five bacterial strains such as Enterococcus faecalis (ATCC - 29212), Enterobacter aerogens (MTCC-111), Staphylococcus aureus (ATCC-25923), Proteus vulgaris (MTCC-1771) and E. coli (ATCC 25922) for antibacterial activities. The highest zone of inhibition was observed against Staphylococcus aureus at 5mg concentration at stations I and III and station II showed highest zone of inhibition against Enterobacter aerogens at 5mg concentration. The lowest zone of inhibition was observed against E.coli at 1.25mg concentration in all three stations. No inhibition zone was observed against Enterococcus faecalis, and Proteus vulgaris in all the concentration.The present study indicates that the crab can be used as an antibacterial for many pathogens.**

Key words: Portunu pelagicus, Enterococcus faecal, Enterobacter aerogens, Staphylococcus aureus , Proteus vulgaris and E. coli.

1. INTRODUCTION

Antibiotics are one of the most important weapons in fighting bacterial infections and have greatly benefited the health quality of human life since their introduction (Sarkar et al., 2003). In recent years one of the more alarming conditions of clinical microbiology is high risk of antibiotic resistance which is increasing day by day among pathogens. Now this increase in resistance is a global problem, no country is immune to this condition and all major bacterial pathogens have acquired resistance to at least one or more drugs (Schito, 2002). As resistance is increased, patients are on high risk of severity because of untreated pathogens (Kerr, 2005).

The ocean serves just not only as the source of antibiotics but indeed a reservoir of other bioactive compounds too. Invertebrates represent the most diverse taxon of animals on the planet, accounting for more species than all other animals combined. Invertebrates have to rely on the innate immune processes to combat pathogens which closely resemble the innate immune system of the vertebrates. As a result, these animals have developed various competent strategies to defend their lives against invading pathogens (Jiravanichpaisal et al., 2006).

Crabs, among numerous other invertebrates are considered as an essential shell fishery product (Nalan et al., 2003). In crustaceans, the antimicrobial substances are considered to be a main component of innate immunity (Smith and Chisholm, 2001). Crabs are the very good resource of antimicrobial proteins with wide range of antimicrobial properties. Antimicrobial peptides are a major component of the innate immune defense system in invertebrates (Tincu and Taylor, 2004).

2. MATERIALS AND METHODS

2.1. Collection and Preparation of extract

In the present study the animals (P. pelagicus) were collected from three stations (Kanyakumari- Station I, Therespuram- Station II and Rameshwaram- Station III) by trawl catch, kept in ice and transferred to the laboratory within 24 hours. For removing mud, algae and barnacles stuck to external skeleton, crabs were washed with fresh sea water. The shells were removed and the tissues were then dried in hot air oven at 56oC for 48 hours. The dried tissue was immersed in 10% AR grade methanol for 10 days at room temperature. After filtration with Whatman No.1 paper, the methanol extract was reduced by vacuum evaporation. The extract residue was resuspended in 20 ml of 100% A.R grade methanol. The methanol soluble extracts were dried and solidified in distilled and deionized water. Different concentrations of extracts were prepared and stored at 0oC for further use.

2.2. Bacterial cultures

Five bacterial strains such as Enterococcus faecalis (ATCC - 29212), Enterobacter aerogens (MTCC-111), Staphylococcus aureus (ATCC-25923), Proteus vulgaris (MTCC-1771) and E. coli (ATCC 25922) were used for antimicrobial activities. (All the bacterial strains were clinical isolates, obtained from

the Microbiology department of Madras Medical College, Chennai).

2.3. Inoculum preparation for bacteria

Nutrient broth was prepared and sterilized in an autoclave at 15lbs pressure for 15 min. All the five bacterial strains were individually inoculated in the sterilized marine broth and incubated at 37oC for 24hour. Nutrient agar (Himedia) was prepared, sterilized in an autoclave at 15lbs pressure for 15 min and poured into sterile petridishes and the 24 hour old bacterial broth cultures were inoculated in the petridishes using a sterile cotton swab.

2.4. Antibacterial assay

The antibacterial activity was carried out by following agar well diffusion method (Perez et al., 1990). The sterilized nutrient agar medium (Himedia) was transferred aseptically into each sterilized petriplates. The plates were left at room temperature for solidification. In each plate, a single well of 6mm diameter was made using a sterile borer. The extracts were freshly reconstituted with suitable solvents and tested at various concentrations (1.25, 2.5 and 5 mg/100µl/well). Each well was loaded with 0.1ml of extract using a micropipette. Chloramphenicol was used as a control. The plates were incubated for 24 h at 37°C.

Inhibition zones were measured and recorded. The experiment was done three times for confirmation of activity and the mean values are presented.

3. RESULTS

3.1. Antibacterial activity

The antibacterial activity of methanol extract of the crab Portunus pelagicus collected from station I is given in plate 1 and in Fig 1. Antibacterial activity was recorded against E. coli at three different concentrations. At 1.25 µl concentration the extract showed 11 mm inhibitory zone. At 2.5 µl, an inhibition zone of 12 mm was observed and at 5 µl concentration, the inhibition zone of 14 mm (maximum antibacterial activity) was observed. For Enterobacter aerogens, the zone of inhibition at 1.25 µl was observed to be 12 mm, for 2.5 µl, it was observed to be 14 mm and for 5 µl, it was observed to be 17 mm. For Staphylococcus aureus, the zone of inhibition at 1.25 µl was observed to be 13 mm, for 2.5 µl, it was observed to be 16 mm and for 5 µl, it was observed to be 20mm.

The methanol extract of the crab tissue (Station II) showed activity with the inhibition zones ranging from 11 mm to 18 mm. Antibacterial activity was recorded against E. coli at three different concentrations. At 1.25 µl concentration the extract showed 11 mm inhibitory zone. At 2.5 µl, a inhibition zone of 14 mm was observed and at 5 µl concentration, the inhibition zone of 18 mm (maximum antibacterial activity) was observed. For Enterobacter aerogens, the zone of inhibition at 1.25 µl was observed to be 13 mm, for 2.5 µl, it was observed to be 15 mm and for 5µl, it was observed to be 18 mm. For Staphylococcus aureus, the zone of inhibition at 1.25 µl was observed to be 12 mm, for 2.5 µl, it was observed to be 13 mm and for 5 µl, it was observed to be 15 mm.

The methanol extract of Portunus pelagicus (Station III) showed activity with the inhibition zone ranging from 18-19 mm. Antibacterial activity was recorded against E. coli at three different concentrations. At 1.25 µl concentration the extract showed 10 mm inhibitory zone. At 2.5 µl, a inhibition zone of 13 mm was observed and at 5 µl concentration, the inhibition zone of 16 mm (maximum antibacterial activity) was observed. For Enterobacter aerogens, the zone of inhibition at 1.25 µl was observed to be 11 mm, for 2.5 µl, it was observed to be 13 mm and for 5 µl, it was observed to be 15 mm. For Staphylococcus aureus, the zone of inhibition at 1.25 µl was observed to be 12 mm, for 2.5 µl, it was observed to be 14 mm and for 5 µl, it was observed to be 17 mm. No inhibition zone was observed against Enterococcus faecalis, and Proteus vulgaris in all the concentration at all the three stations.

Plate 1: Antibacterial activity of extract (Station I)

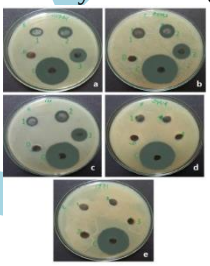


Plate 2: Antibacterial activity of extract (Station- II)

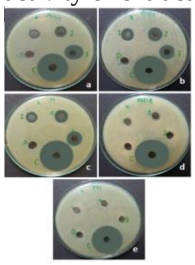
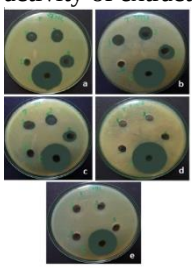


Plate 3: Antibacterial activity of extract (Station - III)



1- 1.25 mg; 2- 2.5 mg; 3- 5.0 mg;
C- Positive Control (Chloramphenicol);
D- Negative Control (DMSO)

a - E.coli(ATCC 25922)
b- Staphylococcusaureus (ATCC-25923),
c- Enterobacteraerogens (MTCC-111),
d- Enterococcusfaecalis (ATCC - 29212),
e- Proteus vulgaris (MTCC-1771)

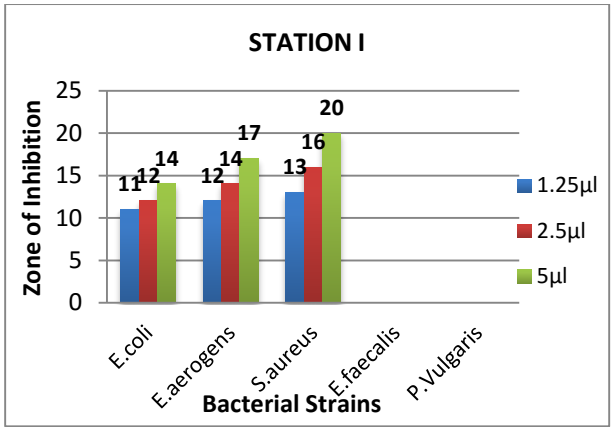


Fig 1: Antibacterial activity of methanol extract of Crab P.pelagicus (Station I)

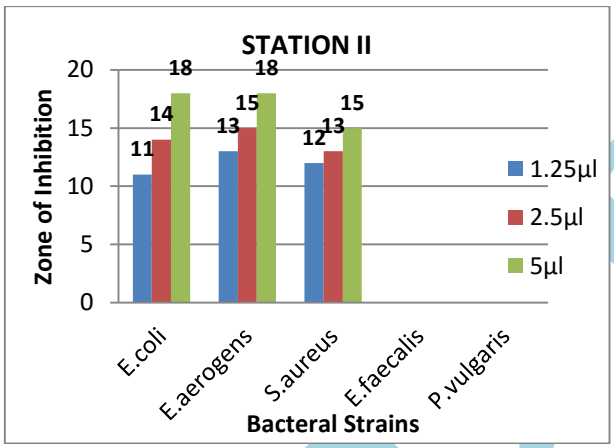


Fig 2: Antibacterial activity of methanol extract of Crab P.pelagicus (Station II)

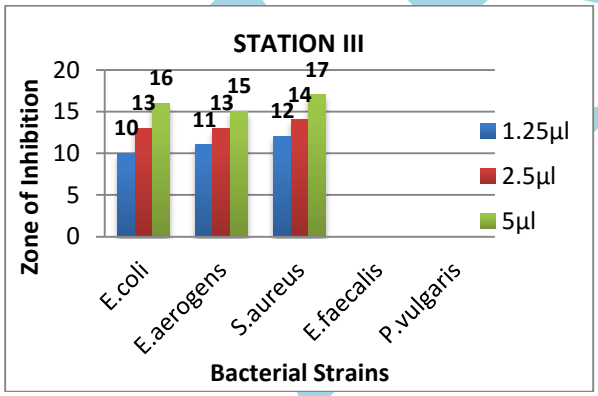


Fig 3: Antibacterial activity of methanol extract of Crab P.pelagicus (Station III)

5.4. DISCUSSION

Antibacterial activity has previously been described in a wide range of crustacean species. In most of the species studied, only the haemolymph and haemocytes have been tested for activity. The present study demonstrated the presence of antibacterial factors in the tissues. The isolated extract from the crab tissue collected from station I showed antibacterial activity against E. coli at three different concentrations. E. coli showed the highest zone of inhibition (14mm) at 5µl concentration of methanol extract, Staphylococcus aureus showed 20mm, the highest zone of inhibition at 5µl concentration and in Enterobacter aerogens 17mm of highest zone of inhibition was observed. The lowest zone of inhibition was reported at 1.25µl, 2.5µl and 5µl concentration of the extract against E.coli, Staphylococcus aureus and Enterobacter aerogens. Similar result was reported by Prakash et al., 2011. He studied the antimicrobial activity of haemolymph collected from freshwater crab, Paratelphusa hydrodromous against five bacterial species namely E.coli, K. pneumonia, P. mirabilis, P. aeruginosa and S. aureus. The haemolymph showed a significant or more effect in controlling the growth of E. coli with an inhibition zone of 17 mm which is more than the positive control. Next to E. coli, K. pneumonia having an inhibition zone of 16 mm in diameter.

Lakshmi et al. (2015) studied the antibacterial activity in the fresh water crab Callinectes sapidus. The methanol extraction showed highest antimicrobial activity against E. coli (32.16 ± 0.28 mm zone of inhibition) and minimum zone of inhibition was observed in Aeromonas sp. and Proteus sp. (18.0 ± 0.5mm). The antimicrobial activity of diethyl ether extract was very effective against Pseudomonas sp and the minimum zone of inhibition was observed in Klebsiella sp. The antimicrobial activity against Aeromonas sp. and Bacillus sp. was found to be 21.16 ± 0.28mm. These findings corroborate the results of the present study.

In station II, the maximum antibacterial activity (10mm, 15mm, 18mm) was observed at 5µl concentration against E.coli, Staphylococcus aureus and Enterobacter aerogens. Maximum antibacterial activity (11mm, 12mm, 13mm) was recorded at 1.25µl concentration against E.coli, Staphylococcus aureus and Enterobacter aerogens. A similar result was observed with the haemolymph of some brachyuran crabs against clinical pathogens. Antimicrobial effect of six brachyuran crabs revealed that the maximum antibacterial effect of crude haemolymph is shown by Dromia abrolhosensis against E.coli and the minimum is shown by the crab S.serrata crab against Klebsiella oxytoca (Ravinchandran et al., 2009). Sivasubramanian, 2010 reported the antimicrobial activity from the haemolymph of the crab Ocypode macrocera. Maximum zone of inhibition was exhibited by methanolic extract hemolymph against S. typhi. But the bacteria Pseudomonas aeruginosa, Shigella flexineri, V. cholerae and S. aureus were insensitive to all extract of the haemolymph.

The methanol extract (Station III) showed activity with the inhibition zone ranging from 18-19 mm. The highest zone of inhibition (16mm, 17mm and 15mm) was observed at 5µl concentration against E.coli, Staphylococcus aureus and Enterobacter aerogens. The lowest zone of inhibition (10mm, 12mm and 11mm) was shown against three bacterial strains at Station III. No inhibition zone was observed against Enterococcus faecalis, and Proteus vulgaris in all the concentration but in contrast Haug et al., (2002) studied the antibacterial activity in different body-parts of Pandalus borealis (northern shrimp), Pagurus bernhardus (hemit crab), Hyas araneus (spider crab) and Paralithodes camtschatica

(king crab). However, the major activity was mainly located in the haemolymph and in the haemocytes.

Yedery and Reddy, (2009) studied the antibacterial activity of protein isolated from Scylla serrata against gram positive and gram negative bacteria. The Gram negative bacteria (E. coli and P. aeruginosa) are more susceptible than Gram positive ones tested (S. aureus and S. pyogenes). Varadharajan and Soundarapandian, (2013) reported the antibacterial activity of crab shell extracts against human pathogenic bacteria and the results indicated that, the maximum inhibition zone was recorded in gram positive S. aureus, S. epidermidis and B. subtilis and minimum inhibition zone was recorded in gram negative P. aeruginosa, P. mirabilis.

Crabs are the wonderful resource of antimicrobial proteins with wide range of antimicrobial properties. The results of the present study indicate that the antibacterial factors are also produced in crustacean tissues other than in haemolymph/haemocytes. The antibacterial activity might be due to factors of the innate immune system. So the animal can be used as an antimicrobial agent for many different pathogens.

CONCLUSION

The present study indicates that the crab would be a good source of antimicrobial agent for the search of new substances for drug development

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