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RESEARCH ARTICLE

ANTIMICROBIAL ACTIVITIES OF THE SHELL OF THE CRAB PORTUNUS PELAGICUS (LINNAEUS, 1758) FROM THOOTHUKUDI COAST

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ABSTRACT

The present investigation has been undertaken to find out the antimicrobial activities of the selected crustacean Portunus pelagicus. Antimicrobial activity of the whole body shell of crabs were tested against five bacterial strains and four fungal strains by disc diffusion method. Among the crab antibacterial activity of shell of Portunus pelagicus showed the best activity against Streptococcus (7mm) and antifungal activity of ethanol shell extract showed the maximum activity against *Aspergillus flavus*. The result of present study revealed that ethanol extract of whole body shell of P. pelagicus showed the best antibacterial and antifungal activity.

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INTRODUCTION

Ocean is a vast potential for a huge number of novel chemicals that may be useful for finding drugs with greater efficacy and specificity for the treatment of many human diseases (Bergmann and Feeney, 1951; Faulkner, 2001). So far, more than 10,000 compounds have been isolated from marine organisms (Proksch *et al*., 200). With hundreds of near compounds still being discovered every year. Crabs are decapods crustacean and are the essential part of macro fauna. Crabs (Brachyura) holds a prosperous diversity and more than 5000 species belonging to700 genera have been identified worldwide. Crabs are one of the extremely diversified and leading groups among crustaceans. These crustaceans such as crabs are considered as healthy food for humans because they contain high quality protein and less amount of fat. Crabs are the fundamental parts of the ecosystem; they are supposed to be good for human consumption and taken as food in many countries. Both fresh water crabs and marine crabs are consumed. India is one of the major contributors of marine crustaceans in the world market. The crab fishery in India is fast developing and there is a vast scope for crab meat, both national and international markets (Manisseri Mary and Radhakrishnan, 2003). Crabs are commercially important and fetch high price as there is a rapidly expanding demand for crab meat both in local and international market. Crabs are found in all type of environment. The evolution of antibiotic resistant pathogenic bacteria has stimulated the search for alternative antimicrobial agents from natural source. Many antimicrobial peptides show a high specificity for prokaryotes and a low toxicity for eukaryotic cells and their mode of action is considered unlikely to lead to development of resistance. These properties have favored their investigation as potential new antibiotics (Bax *et al*., 2000). (Sharmila Joseph *et al*., 2014) Conducted investigational study in order to isolate and identify bacterial and fungal pathogens, from lesioned carapace and limp of the fresh water crab *Barytelphusa cunicularis*, living in the paddy fields of Mananthavady, Wayanand, and kerala. They used pour plate method to isolate bacterial flora and they made phenotypic identification based on morphological characteristics and biochemical analysis. Therefore, there is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action (Immanuale *et al*., 2012). Preyanat Vongchan *et al*. (2003) studied chitosan from marine crab shell in order to find out its anticoagulant activity. Crabs belongs to phylum Arthropoda and subphylum Crustacea. It has a matchless position in evolution. This animal has lost of bioactive materials which unique functions. (Ahmed *et al*., 2012) Extracted compounds from crab shell so as to investigate the antitumor effect of the extract. They used four different extracts derived from crab shell.

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MATERIALS AND METHODS

Collection and preparation of extracts

In the present study the Blue crab *P. pelagicus* were collected from the intertidal area of Gulf of Mannar, thoothukudi coastal region were purchased from the local market. The freshly collected samples were cleaned and washed with fresh sea water to remove impurities. The crabs were kept inside plastic bags and sealed properly inorder to avoid external microbial contaminants. The samples were stored in 200C refrigerator for storage before use.

Preparation of shell extracts

The shells were broken and the soft tissues were removed and washed thoroughly with distilled water. And shell was collected and dried in sunlight after that it was prepared in powder form. Approximately 5g of shell powders were immersed separately into Benzene, Methanol, Ethanol, Chloroform and Hexane solvants and they were cold steeped at -18oc. The extracts from each solvent were filtered twice using Whatman No.1 filter paper. Samples were centrifuged at 5000 rpm for 15 min in rotary evaporator. And the precipitate was collected and it was stored at for further use.

Microbial strains used

Antimicrobial activity of *Portunus pelagicus* was determined against five bacterial strains Viz, Streptococcus, *E.coli*, Citrobacter, Bacillus sps and Enterobacter sps. And four fungal strains *Aspergillus flavus, Aspergillus fumigatus, Alternaria alternate* and Cladosporium.

Inoculum preparation for bacterial strains

Nutrient broth was prepared and sterilized in an autoclave at 151bs pressure for 15 minutes. All the five bacterial strains were individually inoculated in the sterilized marine broth and incubated at 370c for 24 hour. The 24 hour old bacterial broth cultures Streptococcus, *E.coli*, Citrobacter, Bacillus and Enterobacter sps were inoculated in the petridishes using a sterile cotton swab.

Inoculum preparation for fungi

Sephadex Agar (Himedia) broth was prepared and sterilized in an autoclave at 151bs pressure for 15 minutes. The sterilized Sabouraud agar was poured into sterile petridishes and incubated at 370c for three days. The fungal strains *Aspergillus flavus, Aspergillus fumigatus, Alternaria alternate* and Cladosporium. Was inoculated in the broth using a sterile cotton swap and incubated at 370c for 72 hours.

Antimicrobial assay

The spectrum of antibacterial and antifungal activity were studied by using the techniques described by (Bauer *et al*., 1996). The sterilized nutrient agar medium (Himedia) was transferred aseptically into each sterilized petriplates. The plates were left at room temperature for solidification and the disc (10mm) were prepared. The extracts were freshly reconstituted with suitable solvents and tested as various concentrations (10, 25 and 50µl) were added to the each disc. The plates were incubated overnight at 370c. Microbial growth was determined by measuring the diameter of the zone of inhibition. For each strain a control was also maintained where pure solvents were used instead of extract. The experiment was done three times for confirmation of activity.

RESULTS

Antibacterial activity of shell extracts

The shell extracts of *P.pelagicus* were screened for antibacterial and antifungal activity against five human pathogens were represented in the plates (1&2) and Figure (1&2) From the five solvent extracts of shell of *P. pelagicus* the solvents Benzene, and Ethanol showed the best activity against Streptococcus and Bacillus sps (7mm) and the lowest activity was found in *E.coli* and Enterobacter sps (3mm).

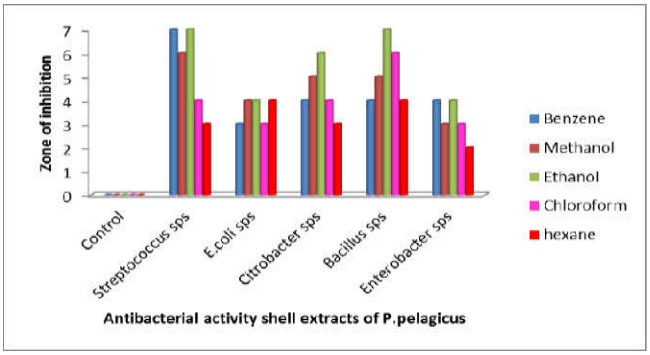


Figure 1. Antibacterial activity of shell extracts of *Portunus pelagicus* against human pathogens

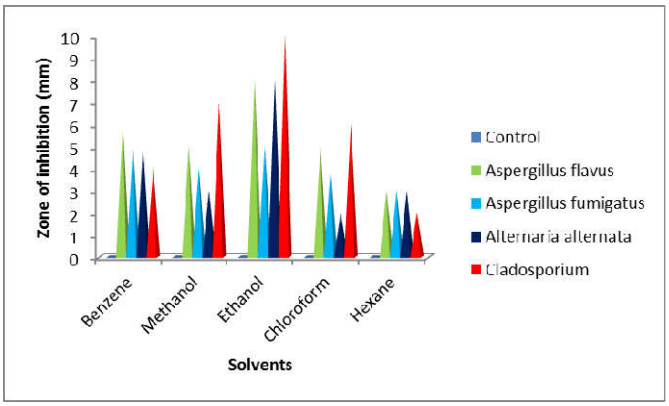
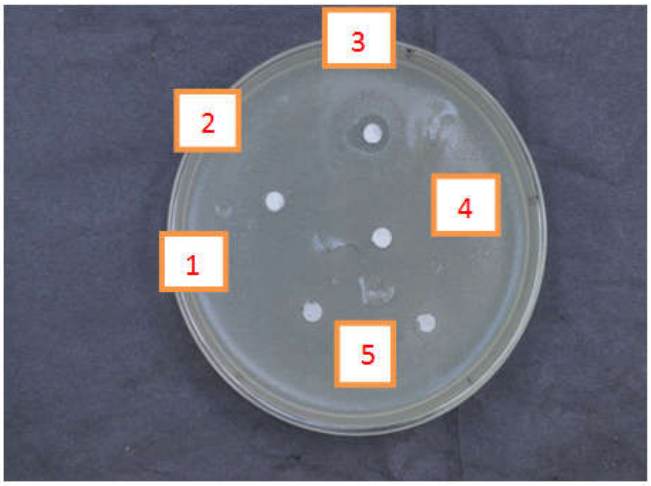


Figure 2. Antifungal activities of shell extract of *Portunus pelagicus* against human pathogens



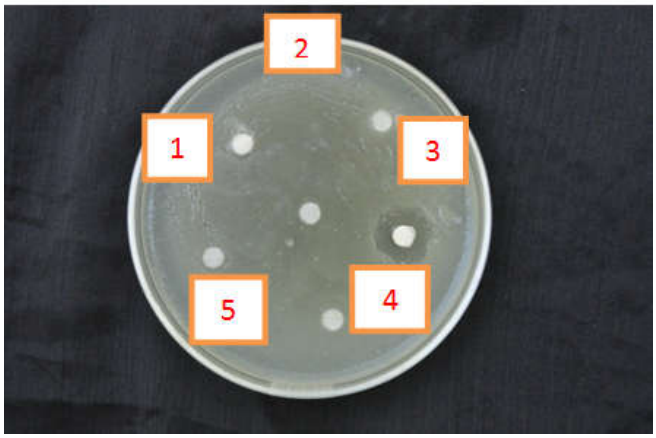
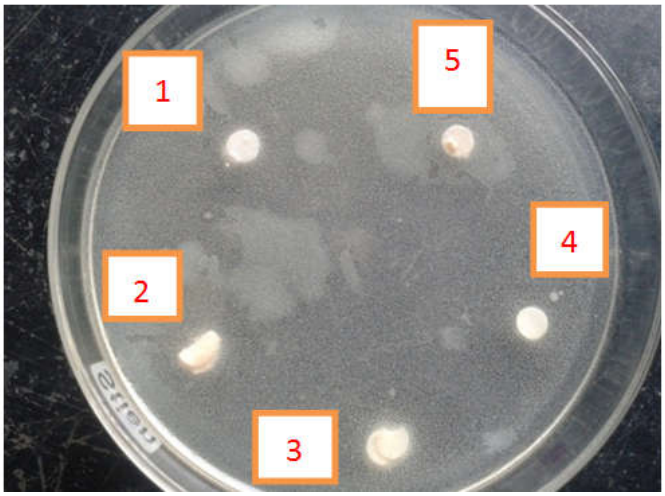


Plate 1. Antibacterial activity of *Portunus pelagicus* (Shell extracts)



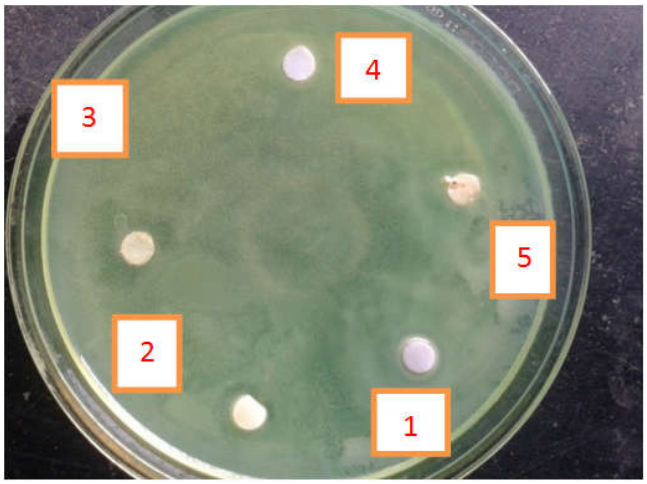


Plate 2. Antifungal activity of *Portunus pelagicus* (Shell extracts)

Antifungal activity of Shell extracts

Antifungal activity of shell extracts showed that the extracts from ethanol showed a high antifungal activity against four human pathogens was represented in the plates. From the five solvent extracts of shell of *Portunus pelagicus,* Ethanol showed the best activity against *Aspergillus flavus, Aspergillus fumigatus, Alternaria alternata*, and Cladosporium (16mm, 10mm) and lowest activity was found in the solvent Chloroform against the strain *Alternaria alternata* (2mm). Plate(2) and figure (2). In the present investigation Ethanol, Chloroform, and Benzene extracts of *P.pelagicus* showed good result with *Aspergillus flavus* (9mm and 8mm), *Aspergillus fumigatus* (8mm and 7mm).

DISCUSSION

In recent years great attention has been paid to study the bioactivity of natural products and their potential pharmacological utilization. Crutaceans are a native of aquatic ecosystem and so inhabiting the harmful effect of microbial growth. A microbial infection has been the major concern of aquaculturist worldwide. The extracts shell of crab shows good activity against microbial strains. In the present investigation, different extract of *P.pelagicus* samples were evaluated against five different pathogenic bacterial and four fungal strains summarized by comparative antimicrobial effect of six brachyuran crabs revealed that the maximum antibacterial effect of crude tissue is shown *Dromia aprolhensis* against *E.coli* and the minimum against the *Scylla serrata* crab against *K.oxytoca*. (Rameshkumar, 1798) The crab haemolymph showed antimicrobial activity against a range of different pathogenic strains of both gram positive and gram negative bacteria. The results suggest that brachyuran crabs were not involved in the economy of finfish resources. It can also produce antibacterial substances instantly to combat bacterial infection. Similar result was observed in the haemolymph of some mangrove crabs against clinical pathogens (Veeruraj *et al*., 2008). Among the five solvent extracts of shells of *P.pelagicus*, the solvents, Ethanol showed the best activity against *Aspergillus flavus*, *Aspergillus fumigatus*, *Alternaria alternata,* and Cladosporium (16mm, 10mm) and lowest activity was found in the solvent Chloroform against the strain *Alternaria alternata* (2mm). In the present investigation Ethanol, Chloroform, and Benzene extracts of *P. pelagicus* showed good result with *Aspergillus flavus* (9mm and 8mm), *Aspergillus fumigatus* (8mm and 7mm).

Conclusion

In the present study it has been recorded that, a wide spectrum at antimicrobial activity is found in almost all the solvents tested and these results indicates that Crustaceans were good source for search of new substances for drug development. From this study, we conclude that the whole body shells of crabs can be used as an antimicrobial agents for many different pathogens and would replace the existing inadequate and cost effective antibiotics. Commercial antibiotics are highly effective to kill the bacterial and fungal pathogens involved in common infection. Ethanol extracts shell of crabs used in the present study showed significant antimicrobial activity compare with other solvent extraction.

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