Antimicrobial Activity of the Tissue Extracts of *Menippe rumphii* (Fabricius, 1798)

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Abstract : The present investigation was undertaken to find out the antimicrobial activity of different tissue extracts of the crab *Menippe rumphii* against four bacterial strains and two fungal strains by disc diffusion method. Two positive control amikasin and flucanozole was also used among the different tissue extracts, antibacterial activity of ethanolic extracts of egg from *Menippe rumphii* showed the highest activity against *Enterobacter* and *Proteus mirabilis.* The results of present study revealed the ethanolic extract of different tissues of *Menippe rumphii* showed the best antimicrobial activity.

Keywords : *Menippe rumphi,* Tissue extracts, antibacterial, *Enterobacter, Proteus mirabilis*.

I. INTRODUCTION

Ocean offers a wide biodiversity of fauna and flora which is estimated to be over 5,00,000 species or more than double of the land species (Kamboj, 1999). Microbial population in seawater and sediments may be as high as 10^6 and 10^9 per milli litter, respectively (Austin, 1988). Marine invertebrates are constantly exposed to high concentrations of the pathogens. The survival of these organisms depends on efficient antimicrobial mechanisms to protect themselves against microbial infections (Soodabeh Kazemi *et al*., 2016). When the pathogens enter in to the body, a complex interaction of innate humoral and cellular immune reactions is induced in both tissues and haemocoel, which results in a fast elimination of microorganisms (Bulet *et al.,* 1999). Humoral immunity in marine invertebrates is mediated by antimicrobial agents present in the blood cells and plasma (Wright, 1981) along with reactions such as hemolymph coagulation and melanisation (Soderhall *et al*., 1996; Tayler *et al*., 1997).

Antimicrobial activity has been detected in several decapod crustaceans, including lobster, crabs, shrimps and freshwater cray fish (Stewart *et al*., 1972; Noga *et al*., 1996). Marine crabs are rich sources of new antibiotics (Ravichandran *et al*., 2010) but only few studies were carried out on the bioactivity of crustaceans (Prakash *et al*., 2011; Ravichandran *et al*., 2010; Packia Lekshmi *et al*., 2015).

Recent research findings suggest that marine crabs are a potential source of new antibiotics for pharmaceutical development. However the marine organisms recognized as a potential source of biologically active substances, is largely unexplored. Hence, a broad based screening of marine crabs for bioactive compounds has become a necessity. Kanyakumari is a rich source of marine reserves since this study focused on the antimicrobial activity of the marine crab, *Menippe rumphii* commonly found in the coast of Kanyakumari District, Tamilnadu, India.

2. MATERIALS AND METHODS

Animal collection and preparation of tissue extract

Live specimens of the crabs were collected from Kanniyakumari coast, Tamilnadu, India. Hemolymph was collected by cutting each walking leg and the exuding hemolymph was allowed to fall directly into plastic tubes kept on ice. To remove hemocytes, the hemolymph was centrifuged at 2000 rpm for 15 minutes at 4 °C. The supernatant were collected and stored in the freezer at -20 °C until use.

To prepare tissue extract of *Menippe rumphii*, adult healthy specimens were dissected the tissues were removed weighed and rinsed twice in cold saline (0.9%). Extracts of the different tissues were prepared as per the method of Abubakar *et al.*, (2012). In this study acetone and ethanol are used as the solvents for extraction of tissues. One gram of different tissues (hepatopancreas, gills, muscles, egg and mantle) were homogenized and extracted with 10 ml of acetone/ethanol. Then the crude extract was centrifuged (12000 g for 5 minutes). The extract was filtered through Whatman No. 1 filter paper. The filtrate was used for the antimicrobial activity using agar disk diffusion method.

Test microorganisms

Test microorganisms such as *Enterobacter*, *Proteus mirabilis*, *Bacillus subtilis*, *Aspergillus niger* and *Aspergillus flavus* used in this study were obtained from Vivek Laboratory, Nagercoil, Kanyakumari District, Tamilnadu, India.

Antibacterial assay

The antimicrobial and antifungal activity was studied using standard techniques (Bauer *et al*., 1996). The antimicrobial activity was evaluated in terms of the diameter of the zone of inhibition surrounding disc using a scale and was recorded.

3. RESULTS

In the present study the antibacterial and antifungal activities of ethanol and acetone extracts of the egg, hepatopancreas, gills, hemolymph, muscles and mantle against the four bacterial strains and 2 fungal strains were evaluated. The results of antimicrobial activity of *Menippe rumphii* are presented in table 1a and b and figure 1-3. Among the tested samples, the ethanolic extracts of *Menippe rumphii* egg showed maximum antimicrobial activity (15 mm) against *Enterobacter* and it followed by *Proteus mirabilis* (12 mm). No activity was recorded against *B. subtilis, E. coli, A. niger* and *A. flavus*

Table 1a. Antimicrobial activity of ethanol extracts of different tissues of *Menippe rumphii*

Ethanolic extracts of *Menippe rumphii*

	Zone of inhibition (mm)					
Organisms	1	2	3	4	5	6
Enterobacter	4	15	8	5	6	7
Proteus mirabilis	4	12	5	7	5	5
Bacillus subtilis	-	-	4	4	4	4
Escherichia coli	-	-	-	-	-	-
Aspergillus niger	-	-	-	-	-	-
Aspergillus flavs	-	-	-	-	-	-

1-Hemolymph, 2-Egg, 3-Hepatopancreas,4-Muscle, 5-Gills, 6-Mantle

Table 1b. Antimicrobial activity of acetone extracts of different tissues of *Menippe rumphii*

Acetone extracts of *Menippe rumphii*

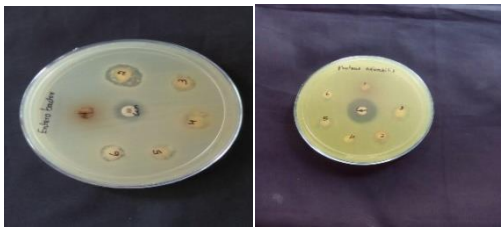
Organisms	1	2	3	4	5	6
Enterobacter	-	-	-	-	-	-
Proteus mirabilis	-	-	-	-	-	-
Bacillus subtilis	-	-	-	-	-	-
Escherichia coli	-	-	-	-	-	-
Aspergillus niger	-	-	-	-	-	-
Aspergillus flavs	-	-	-	-	-	-

1-Hemolymph, 2-Egg, 3-Hepatopancreas,4-Muscle, 5-Gills, 6-Mantle

The ethanolic extracts of hepatopancreas showed maximum activity (8 mm) against *Enterobacter* and lowest activity (4 mm) was observed against *B. subtilis*. No zone of inhibition was observed against *E . coli, A. niger* and *A. flavus*. The hemolymph of *Menippe rumphii* showed maximum inhibition zone (4 mm) against *P. mirabilis* and *Enterobacter* (4mm). The hemolymph of *Menippe rumphii* failed to show any antibacterial and antifungal activity against *E.coli, B. subtilis, A. niger* and *A. flavus.* The etanolic extracts of gills showed activity against *Enterobacter* (6 mm) followed by *Proteus mirabilis* (5 mm) and *Bacillus subtilis* (4 mm) no zone of inhibition was observed against *E.coli, Aspergillus niger* and *Aspergillus flavus*. The ethanolic extract of muscle observed the maximum inhibitory activity against *Proteus mirabilis* (7 mm) and followed by *Enterobacter* (5 mm) and *Bacillus subtilis* (4 mm). No activity was recorded against *E.coli, Aspergillus niger*, and *Aspergillus flavus*. Mantle of showed 7 mm and 6 mm against *Enterobacter* and *Proteus mirabilis* and 4 mm zone was observed against *B. subtilis* and no zone was recorded against *E. coli, Aspergillus niger* and *Aspergillus flavus*. The acetone extracts did not show any antimicrobial activity against all tested organisms.

Plate1: Antibacterial activity of ethanolic extract *Menippe rumphii* against *Enterobacter* (A), *Proteus mirabilis* (B) and *Bacillus subtilis* (C)

A B



C

4. DISCUSSION

The presence of antimicrobial compounds has been reported in crustacean species including the crabs *Carcinus maenas* (Schnapp *et al.,* 1996), *Callinectes sapidus* (Khoo *et al.,* 1999). *Charybdis lucifera* (Ramesh Kumar *et al.,* 2009), *Scylla serrata* (Krishnamoorthi *et al.,* 2016). In the present study the ethanolic extract of *Menippe rumphii* egg showed highest antimicrobial activity against *Enterobacter* (16 mm) and *Proteus mirabilis* (14 mm). Antimicrobial activity has also been studied in egg extracts of echinoid *Paracentrotus lividus* (Stabili *et al*., 1996) and the asteroid *Marthasterias glacialis* (Stabili and Pagliara, 1994), *Diadema setosus* (Marimuthu *et al*., 2015). The antibacterial activity of *Menippe rumphii* egg may be due to the presence of antimicrobial compound was shown to be a lysozyme. Lysozyme are a group of enzymes that cleave the glycosidic bonds between N-acetylmuramic acid N-acetylglucosamine in the peptidoglycans that form bacterial cell walls are especially important antibacterial molecules of their bactericidal ability (Archana Injal *et al*., 2016).

The antibacterial activity was detected in the hemolymph of *Menippe rumphii* against *Enterobacter* and *Proteus mirabilis*. It may be due to broad spectrum of antimicrobial compounds where secreted in response to immunization of pathogens. Similar observations were also found in *Tachypleus tridentatus* (Nakamura *et al*., 1988). *Charybdis feriatus, C . lucifera, C . natator, Portunus sanguinolentus, Portunus pelagicus* and *Dromia dehanni* (Anbuchezhian *et al*., 2009). Crab hemolymph contain antimicrobial activity may be due to the factors of innate immune system.

In the present study antimicrobial activity also shown in the hepatopancreas, gills, muscles and mantle of *M. rumphii*. Similar antimicrobial activity work was reported in different body parts of *Pagurus bernhardus* (Hermit crab), *Pandalus borealis, Hyas araneus* and *Paralithodes camtschatica* (King crab) (Haug *et al*., 2002). Antibacterial activity of hepatopancreas has been reported in the *Portunus segnis* (Mona Hajirasouli and Jamileh Pazooki, 2014), *Meretrix meretrix* (Archana Injal *et al*., 2016).

In the present study indicated that the *Menippe rumphii* can be a source of novel antibiotics. The ethanolic extract showed the highest antimicrobial activity against *Enterobacter* and *Proteus mirabilis*. This study revealing the antimicrobial compounds in the tissues of *M. rumphii* will provide an opportunity for the production of new antimicrobial compounds with natural activity as alternative to synthetic antibiotics. In this study antimicrobial activity of *M. rumphii* was done in first time have not been reported earlier. Further work is progress to isolate, purify, identify the bioactive compounds in the *M. rumphii* extracts using various analytical methods.

REFERENCE

Abubakar, L., Mwangi, C., Uku, J., Ndirangu, S. 2012. Antimicrobial activity of various extracts of the sea urchin *Tripneustes gratilla* (Echinoidea). African Journal of Pharmacology and Therapeutics, 1 : 19-23.

Archana Injal., Somnath Kshirsagar., Mayuresh Dev., Kshitija Parkar., Abida kazi., Mangesh Chavan., Kulkarni, A. S. 2016. Antimicrobial activity of extracts of hepatopancreas and mucus of bivalve, *Meretix meretix*. European. Journal of Biotechnology and Bioscience. 4 (4) : 36-38.

Anbuchezhian, R. M., Ravichandran, S., Rameshkumar, G. and Ajithkumar, T.T. 2009. Influence of crab hemolymph on clinical pathogens. Advances in Biological Research, 3 (3-4): 104-109.

Austin, B.1988. Marine Microbiology (Book). Cambridge University Press, Cambridge.

Bauer, A.W., Kirby, W.M.M., Sherris, J.C and Turck, M. (1996). Antibiotic susceptibility testing by a standardized single disc method. American Journal of Clinical Pathology, 45 : 493-496.

Bulet, P., C. Hetru, J. Dimaroq and Hofmann, D., 1999. Antimicrobial peptides in insects. Structure and function. Dev. Com. Immunol., 23 : 329-344.

Haug, T., Kjuul, A. K., Sandsdalen, E and Styrvold, O. B. 2002. Antibacterial activity in four marine crustacean decapods. Fish Shellfish Immunol, 12 (5) : 371-85.

Kamboj V.P. 1999. Bioactive agents from the ocean boat In : Somayajulu B. L. K editors. Ocean Science Trends and Future Directions. Indian National Science Academy, New Delhi, 197-227.

Khoo, L., Robinette, D. And Noga, E. 1999. Callenectin, an antibacterial peptide from blue crab, *Callinectes sapidus*, hemocytes. Marine Biotechnology, 1 : 44-51.

Krishnamoorthi, A., Selvakumar, S. And Shanthi, G. 2016. Antimicrobial activity of lectins isolated from the hemolymph of marine crab, *Scylla serrata* (Forskal, 1775), Int. J. Modn. Revs, 4 (11) : 1350-1352.

Marimuthu, K., Gunaselvam, P., Aminur Rahman, M., Xavier, R., Arokiaraj, T., Subramanian, S., Yusoff, F. M., Arshad, A. 2015. Antibacterial activity of ovary extract from sea urchin *Diadema setosum*. European Rev for Medical and Pharmacological Sciences. 19 : 1895-1899.

Mona Hajirasouli., Jamileh Pazooki., 2014. Antimicrobial potential of hemolymph and hepatopancreas of *Protunus segnis* crabs. International Journal of Pharmacy and Pharmaceutical Science, 6 (8) : 601-603.

Nakamura, T., Furanaka, H., Miyata T., Muta, F., Iwanaga, S., Niwa, M., Takao, T., and Shimonishi, Y. 1988. *Tachyplesin* a class of antimicrobial peptide from the hemocytes of the horseshoe crab (*Tachypleus tridentatus*). J. Biol. Chem., 263 : 16709-16713.

Noga, E. J., Arroll, T. A Fan and Zhigin, 1996. Specificity and some physicochemical characteristics of the antibacterial activity from the blue crab *Callinectes sapidus*. Fish shell fish-Immunology, 6(6) : 403-12.

Packia Lekshmi, N.C.J., Brindha Shini, A., Jeeva, S., Rajabrindha, J., Selva Bharath, M. (2015). Synthesis of silver nanoparticles using hemolymph of marine crabs (*Carcinus maenas* and *Ocypode quadrata*) and its influence on clinical pathogens. J. Chem Phrm Res, 7 : 598-606.

Prakash, A. A., Balasubramaniam, S., Gunasekaran G., Prakash, M., Senthilraja, P. 2011. Control of clinical pathogens by the haemolymph of *Paratelphusa hydrodromous*, a freshwater crab, ISRN pharmacol, 1-4. doi : 10. 5402/642768.

Ravichandran, S., Siva Subramanian, K., Anbuchezhian, R.M. 2010. Antimicrobial activity from the haemolymph of the crab *Ocypode macrocera* (H-Milne Edwards 1852). World Appl Sci J. 11 : 578-81.

Rameshkumar, G.T., Aravindhan, T., Ravichandran, S. 2009. Antimicrobial proteins from the crab *Chrybdis lucifera* (Fabricius, 1798). Middle-East J of Scientific Res, 4 (1) ; 40-3.

Schnapp, D., Kemp, G. D., Smith, V. J. 1996. Purification and characterization of a proline rich antibacterial peptide, with sequence similarity to bactenecin-7, from the hemocytes of the shore crab, *Carcinus maenas*. European J of Biochemistry, 240 : 532-39.

Soderhall, K., Iwanaga, S. and Vasta, G.R, 1996. Crustacean immunity, Annual Review of fish Diseases, 2 : 3-23.

Soodabeh Kazemi., Behrooz Heidari., Mehdi Rassa, 2016. Antibacterial and haemolytic effects of aqueous and organic extracts from different tissues of sea urchin *Echinometra mathaei* pathogenic *streptococci*. Int Aquat Res, 8 : 299-308.

Stabili, L and Pagliara, P. 1994. Antibacterial protection in *Marthasterias glacialis* eggs-characterization of lysozyme-like activity. Comp. Biochem Physiol B, 109 : 7019-713.

Stabili, L., Lassagues, M., Pastore, M. 1996. Preliminary study on the antibacterial capabilities of eggs of *Paracentrotus lividus* (Echinidermata : Echinodea) . J. Invertebr Pathol, 67 : 180-182.

Stewart, J.E. and B.M. Zwicker, 1972. Natural and induced bactericidal activities in the haemolymph of the lobster, *Homarus americanus* : products of hemocyte-plasma interactions. Canadian J. Microbial., 18 : 1499-1509.

Taylor, S.W., Kannmerer, B. and Bayer, E. 1997. New induced lysis, Biochemistry, 34 : 4393-401.

Wright, R.K. 1981. Urochordates, Academic Press, Ltd., London, England.2.