Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 5, Issue, 12, pp.4066-4069, December, 2013

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

ANTIBACTERIAL ACTIVITY OF HEMOLYMPH OF SELECTED GASTROPODS SPS COLLECTED FROM KANYAKUMARI COAST AGAINST HUMAN PATHOGENS

***Sharmin Vini, S., Asha, A., Viju, N., Sunjaiy Shankar, C. V. and Mary Josephine Punitha, S.**

Cenre for Marine Science and Technology, Manonmanium Sundaranar University, Rajakamangalam

ARTICLE INFO

Article History:
Received 07th September, 2013
Received in revised form
15th October, 2013
Accepted 29th November, 2013
Published online 25th December, 2013

Key words:
Gastropods,
Haemolymph,
Antimicrobial activity,
Human pathogens.

ABSTRACT

Molluscs constitute the second largest animal phylum, after Arthropods, in terms of number of species. Molluscs are widely distributed throught the world and have many representatives such as slugs, whelks, clams, mussels, gastropods, oysters, scallops, squids and octopods etc. in marine and estuarine ecosystem. The present study was carried out to screen the antimicrobial activity of haemoplymph of selected gastropod species. The antimicrobial activity of the haemolymph sample was carried out against seven human pathogens namely *Bacillus subtilis*, *E.coli Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus* and *Streptococcus mutans*. The maximum zone of inhibition was observed against *Klebsilla pneumonia*, *Bacillus subtilis*, *Salmonella typhi* (8mm) by the haemolymph collected from *Nerita albicilla.* The protein content of the haemolymph sample was estimated using Lowry's method. Variation in protein content of the haemolymph among the gastropod species was observed.

INTRODUCTION

Molluscs are widely distributed throughout the world and have many representatives such as slugs, whelks, clams, mussels,gastropods, oysters, scallops, squids and octopods in the marine and estuarine ecosystem. Many classes of bioactive compounds exhibiting anti-tumor, anti-leukemic, antibacterial and antiviral activities have been reported worldwide (Rajaganapathy *et al*., 2000). Among the molluscs some have pronounced pharmacological activities or other properties which are useful in the biomedical area. It is surprising to find that some of the pharmacological activities are attributed to the presence of polysaccharides particularly sulphated mucopolysaccharide. Antimicrobial peptides are important in the first line of the host defense system of many animal species (Boman, 1995). Haemolymph in marine invertebrates performs many important tasks as it distributes oxygen, nutrients, hormones, components of the immune system and often also vitellogenic and storage proteins. Most of the studies concerning antimicrobial activity includes specific compartments like egg masses, hemolymph or whole body extracts of mollusc (Haug *et al*., 2003). The presence of antimicrobial activity has been reported in the digestive gland, albumin gland, egg masses and purple fluid of the Nudibranch (Minale and Riccio, 1976; Kamiya *et al*., 1989; Yamazaki, 1993). In case of cephalopods, the antimicrobial activity has been reported in the mucous and cuttle bone of the giant snail (Iguchi, 1982; Rajaganapathi, 2001). Other than antimicrobial activity, a novel agglutinin which is responsible for hemagglutination was isolated from the sea hare *A. kurodai*, *A. juliana*, *A. dactylomela* Yamazaki (1993). The potential of marine gastropod as a source of biologically active products is largely explored in India. Therefore, the aim of our present study is to evaluate the antimicrobial activity of the haemolymph of gastropods against different human pathogenic bacterial strains.

***Corresponding author: Sharmin Vini, S.,** Cenre for Marine Science and Technology, Manonmanium Sundaranar University, Rajakamangalam.*

MATERIALS AND METHODS

Collection of samples

Live specimens of gastropod species such as *Nerita albicilla, Purpura bufo, Turbo petholatus, Purpura patula, Purpura panama,* and *Tronchus niloticus* were collected from Colachel, Muttom, and Kanyakumari coastal areas along the Southwest coast of India. They were immediately brought to the laboratory aseptically.

Preparation of haemolymph

Animals having an average weight of 50 g were used for the experiments. Haemolymph was collected by puncturing the pedal blood sinus with a syringe needle. Approximately 5 ml of haemolymph was collected from each animal. Haemocytes and cellular debris were removed by centrifugation at 10,000 ***g*** for 20 min at 4°C. The haemolymph was stored at −20°C until further use.

Antibacterial assay

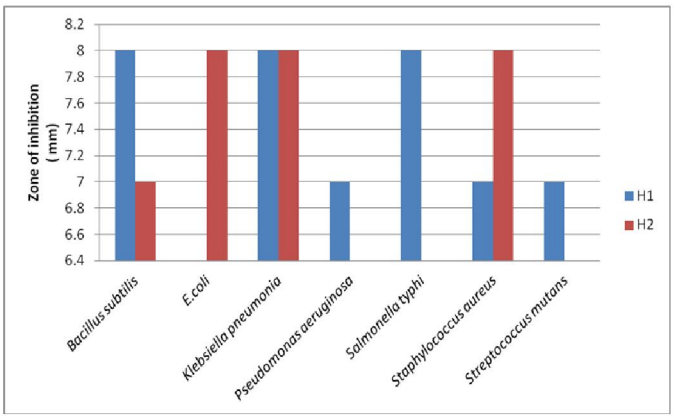
Haemolymph collected from the selected gastropods was tested against human pathogenic bacteria such as *Bacillus subtilis* (strain no.1134), *E.coli* (strain no.1671), *Klebsiella pneumonia* (strain no. 7407) *Pseudomonas aeruginosa* (strain no.6538), *Salmonella typhi* (strain no.733), *Staphylococcus aureus* (strain no.916) and *Sterptococcus mutans* (strain no.1936). Microbial assay was carried out by disc diffusion technique followed by Kelman *et al.,* 2001. Pathogenic bacterial strains were inoculated in sterile nutrient broth and incubated at 37°C for 24 h. Pathogens were swabbed on the surface of the Muller Hinton agar plates and discs (Whatman No.1 filter paper with 5 mm diameter) impregnated with 50 µL of gastropod haemolymph were placed on the surface. The plates were incubated at 37°C for 24 h and the antimicrobial activity was measured accordingly based on the inhibition zone around the disc impregnated with haemolymph.

Estimation of protein concentration

Protein concentration of the sample was determined by the method followed by Lowry *et al*. (1951) using bovine serum albumin (BSA) as a standard. Biuret reagent was used as a colour reactant and concentration was calculated in response to absorbance at 650nm in spectrophotometer.

RESULTS

The haemolymph collected from six different gastropods species namely *N.albicilla, P.bufo, T.petholatus, P.patula, P.panama, T.niloticus* were screened against seven human pathogens, such as *B.subtilis, E.coli, K.pneumonia, P.aeruginosa, S.typhi, S.aureus* and *S.mutans*. The results of the screening test are summarized in Table1 and also graphically represented in Fig1. Among them, the haemolymph collected from *N. albicilla* showed maximum zone of inhibition (8mm) against *Klebsiella pneumonia*, *Bacillus subtilis, Salmonella typhi*. The haemolymph of *P. bufo* showed maximum zone of inhibition (8mm) against *E.coli* and *Staphylococcus aureus* but minimum zone of inhibition was observed against *Klebsiella pneumonia* and *Bacillus subtilis*. No zone of inhibition was observed against human pathogens by the haemolymph collected from the other gastropod sp.



H1- *Nerita albicilla,* H2- *Purpura bufo*

Figure 1. Antimicrobial activity of haemolymph of gastropods against *Bacillus subtilis, E.coli Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella typhi, staphylococcs aureus* and *Streptococcus mutans*

Protein content

Comparison in protein content of the haemolymph of six gastropod species is shown in Fig 2 and Table2. The results of protein content showed a maximum protein content of 15.51% in *N.albicilla* and the minimum protein content of 10.5% was recorded in *P. panama.* The protein content of *P.bufo, T.petholatus, P.patula* and *T. niloticus* were calculated as 12.05, 10.45, 15.38 and 12.09% respectively.

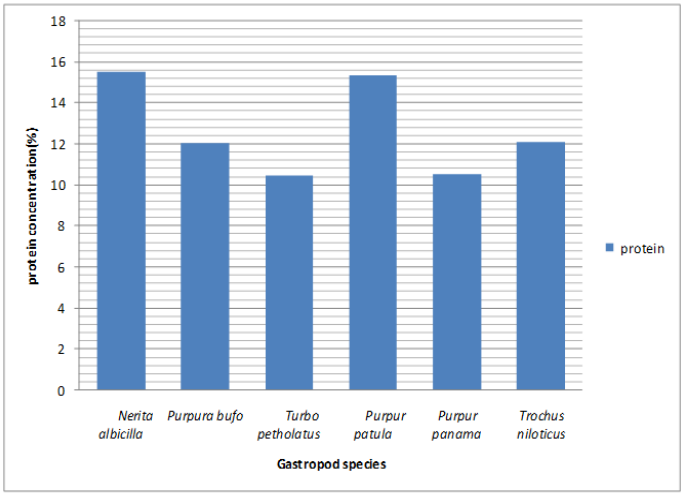


Figure 2. Comparison of protein content in the selected gastropod species

Table 1. Antimicrobial activity of the haemolymph collected from the selected gastropod species

GASTROPOD SPECIES	ZONE OF INHIBITION AGAINST PATHOGENS (mm)						
	Bacillus subtilis	*E.coli*	*Klebsiella pneumonia*	*Pseudomonas aeruginosa*	*Salmonella typhi*	*Staphylococcus aureus*	*Streptococcus mutans*
Nerita albicilla	8±0.86	-	8.16±0.57	7±0	8.33±0.57	7.66±0.57	7±0
Purpura bufo	7±0	8.33±0.28	8±0.86	-	-	8.66±0.76	-
Turbo petholatus	-	-	-	-	-	-	-
Purpura patula	-	-	-	-	-	-	-
Purpura panama	-	-	-	-	-	-	-
Trochus niloticus	-	-	-	-	-	-	-

Table 2. Comparison in protein content of the haemolymph the among six gastropod species

Gastropod species	Percentage of protein concentration (%)
Nerita albicilla	15.51
Purpura bufo	12.05
Turbo petholatus	10.45
Purpur patula	15.38
Purpur panama	10.5
Trochus niloticus	12.09

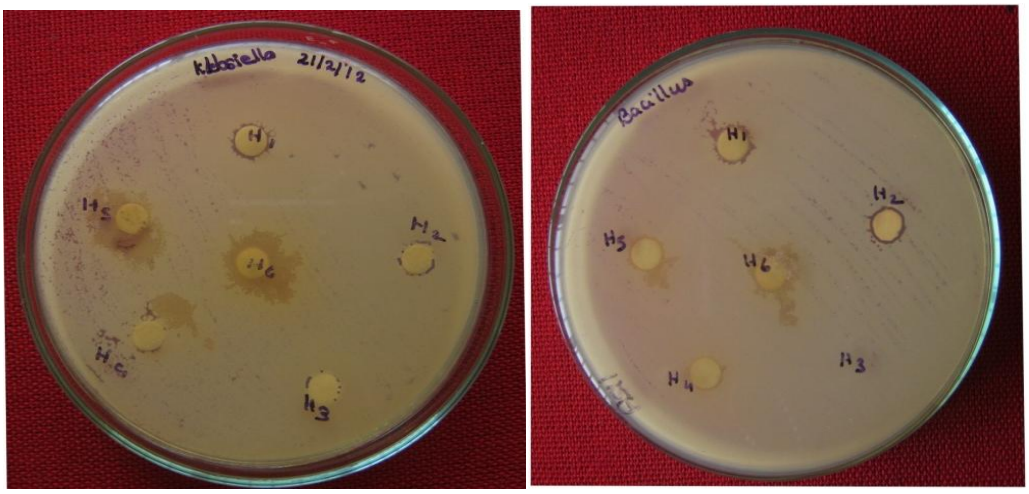


Plate 1. Antipathogenic activity of haemolymph collected from *Nerita albicilla* against *Klebsiella pneumonia* and *Bacillus subtilis*

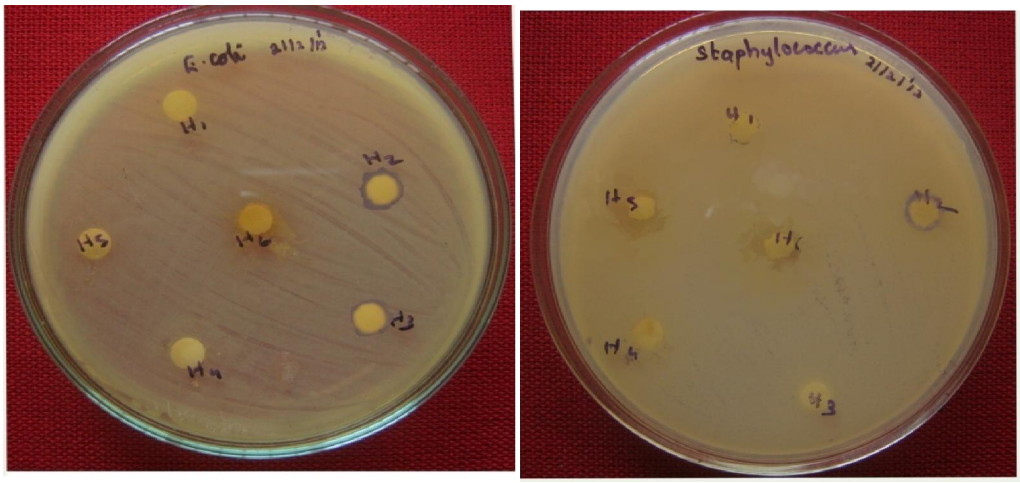


Plate 2. Antipathogenic activity of haemolymph collected from *Purpura bufo* against *E.coli* and *Staphylococcus aureus*

DISCUSSION

There is growing interest in marine natural products or marine secondary metabolites. This field of research receives the attention of investigators from various fields such as marine biology, marine ecology, biochemistry, chemistry, pharmacology and biotechnology. In the industrialized countries, about 25% of all prescription drugs contain active principles that are still extracted from higher plants. In traditional Indian medicine, especially siddha medical preparations, the opercula of gastropods are used as an ingredient to combat different diseases. In the present study, a pronounced antimicrobial activity has been observed against some bacterial strains. In the antimicrobial activity the zone of inhibition 8mm was recorded against *B.subtilis, K.pneumonia and S.typhi*, by the hemolymph collected from *N.albicilla* and the same was recorded against *E.coli, K.pneumonia ans S.aureus*, by the hemolymph collected from *P.bufo*. The presence of antimicrobial activity in mollusca has been reported from the mucus of the giant snail *Achantina fulica* (Kubota *et al*., 1985), from the egg mass and purple fluid of the sea hare *Aplysia kurodai* (Yamazaki *et al*., 1990) and from the body wall of the sea hare *Dolabella aurigularia* (Lijima *et al*., 2003). Kamiya *et al*. (1984) isolated aplysianin-E, an antibacterial glycoprotein from nudibranch Aplysia. Yamazaki *et al*. (1990) have reported the antibacterial activity of the purple fluid of *Aplysia kurodai*. An antibacterial peptide Dolabellanin-A, was identified and characterized from albumin gland of sea hare, *D.auricularia* (Kisugi *et al*., 1992).

Conclusion

Further studies are required to study the nature of proteins present in the haemolymph of tested gastropods. The presence of antimicrobial effect in the tested haemolymph has led to the conclusion that these tested gastropods would help in the development of antibacterial drugs to overcome problem of multidrug resistance to compact human and animal diseases.

REFERENCES

Boman, H. 1995. Peptide antibiotics and their role in innate immunity. *Annu. Rev. Immunol.*, 13: 61-92.

Haug T, Stensverg K, Olsen O, Sandsdalen E, Styrovid O. B 2003. Antibacterial activities in the various tissue of the horse mussel Modiolus. *J.Invertebr.Pathol*, 85:112-119.

Iguchi, S.M., Aikawa, T, Matsumoto, J.J. 1982. Antimicrobial activity of snail mucus mucin. Comp Biochem Physiol A Comp Physiol., 72: 571-574.

Kamiya, H., Muramoto, K and Ogata K. 1984; Antibacterialactivity in the egg mass of a sea hare. *Experientia* 40: 947-949.

Kelman, D., Kashman, E., Roserberg, M., Ilan, I., Ifrach and Loya, 2001. Antimicrobial activity of reef sponge Amphimedon viridis from the Red sea: evidence for selective toxicity. *Aquat. Microb. Ecol*., 24: 9-16

Kisugi, J., Ohye, H., Kamiya H and Yamazaki, M. 1992. Biopolymers from marine invertebrates: XIII. Characterization of an antibacterial protein, Dollabellami-A from albumin gland of the sea hare, *Dollabella auricularia*. *Chem. Pharma. Bull*., 40(6): 1537-1539.

Kubota, Y., Watanabe, Y., Otsuka, H., Tamiya, T., Tsuchiya, T and Matsumoto, J, J. 1985. Purification and characterization of an antibacterialfactor from snail mucus. *Comp Biochem Physiol C*; 82: 345-348.

Lijima, R., Kisugi, J and Yamazaki, M. A. 2003. Novel antimicrobial peptide from the sea hare *Dolabella auricularia*. *Dev Comp Immunol* 27(4): 305-311.

Lowry, O.H., Rosebrough, N.S., Farrand, A.L. and Randall R.J. 1951. Protein measurement with folin phenol reagent, *J. Biol. Chem*.,193, 263-275.

Minale, L., Riccio, R. 1976. Constituents of the digestive gland of the molluscs of the genus *Aplysia* –I.Novel diterpenes from *Aplysia Depilans* .*Tetrahedon Lett*., 31: 2711-2714.

Rajaganapathi, J. 2001. Antimicrobial activities of marine mollusk and purification of anti-HIV protein, Ph.d thesis, India.

Rajaganapathi, J., Kathiresan, K and Sing, T.P. 2000. Purification Anti-HIV protein from purple fluid of the sea hare Bursatella leachii de Blainville. *J. Mar. Biotechnol.*, 4:447-453.

Yamazaki, M. 1993. Antitumor and antimicrobialglycoproteins from sea hares. *Com Biochem Physiol*, 150C (2): 141-146.

Yamazaki, M., Ohye, H., Kisugi, J and Kamiya, H. 1990. Bacteriostatic and cytolytic activity of purple fluid from the sea hare. *Dev. Comp. Immunol*., 14: 379-383.

****