Research Article [Gayathri & Sanjeevi, 5(10): Oct., 2014:3894-3898]
CODEN (USA): IJPLCP ISSN: 0976-7126

INTERNATIONAL JOURNAL OF PHARMACY & LIFE SCIENCES
(Int. J. of Pharm. Life Sci.)

Antipathogenic activity of freshwater Gastropod *Pila virens* (Lamarck, 1822) from Lower Grand Anaicut Reservoir, Tamilnadu

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Abstract

Molluscs are highly delicious seafood and they are also very good source for biomedically imported products. Gastropod crude extract was tested for inhibition of bacterial growth against human pathogenic bacteria. *Pila virens* was screened against five human pathogenic bacteria strains for antibacterial activities. Five species of pathogenic bacteria namely *Klepsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus , Enterococcus faecalis*. Control discs were with water and solvents to assess the effect of water and solvents on pathogens. The plates were incubated at 37 ^{0}C for 24 h the antimicrobial activity was measured accordingly based on the inhibition zone around the disc impregnated with gastropod extract. Ethanol extract of *Pila virens* was found active against five species of pathogens. The inhibition zone ranged from 9 mm to 11 mm. The maximum inhibition zone was 11 mm against *Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecalis.* The minimum zone was 9mm in *Salmonella typhi* and 10mm in *Klepsiella pneumoniae.* Water extract of *Pila virens* zone ranged from 9mm to 10mm. The maximum inhibition zone was 10mm in *Staphylococcus aureus, Enterococcus faecalis* and the minimum inhibition zone was 9mm in *Klepsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi.* Commercial antibiotics are highly effective to kill the bacterial and fungal pathogens involved in common infection. Water, ethanol extracts of gastropods used in the present study showed significant antibacterial activity compare with other solvents extraction. FTIR analysis revealed the presence of bioactive compounds signals at different ranges. It is worthy to note that the product from natural source is good for health and to avoid side effects.

Key-Words: Molluscs, Gastropoda, Antipathogenic activity, *Pila virens*, *Pseudomonas aeruginosa.* FTIR analysis

Introduction

Molluscs are a highly diverse group, in size, in anatomical structure, in behaviour and in habitat (Haszprunar, 2001). Representatives of the phylum live in a huge range of habitats including marine, freshwater and terrestrial environments. In the most of the publications concerning antimicrobial activity in Mollusca, either single body compartment alone, like haemolymph and egg masses, or extracts of whole bodies have been tested for activity (Haug *et al*., 2003). Several of these bioactive natural products provide vital starting materials for the rational generation of libraries of compounds against infectious diseases, cancer and neurological targets, prepared through semisynthesis and biocatalysis (Cooper, 2004).

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Many classes of molluscs exhibits bioactive compounds like antitumor, antileukemic, antibacterial and antiviral properties have been reported worldwide (Petit *et al*., (1987); (Kamiya, *et al*., 1989); Anand *et al*., (2001); Rajagnapathi *et al*.,(2002) among the molluscs some animals exhibited pharmacological activities or other properties which are useful in the biomedical area. Among the molluscs, oysters and mussels are very good source for bioactive compounds considering the importance of the group and paucity of information in this line present study has been undertaken to ascertain the antibacterial activity of extracts from *Pila virens* against various pathogenic bacteria.

Molluscs are highly delicious seafood and they are also very good source for biomedically imported products. 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. Their value in innate immunity lies in their ability to function without either high specificity or memory. Moreover, they are synthesized without dedicated cells or tissues and they can rapidly diffuse to the point of infection. The potential of marine gastropod as a source of biologically active products are largely explored in India.

Material and Methods

Pila virens were collected from the Lower Anaicut Reservoir, Thanjavur District, Tamilnadu (11^0 15' N and 79^0 30' E). Animals were brought to the laboratory, the shells were broken and the tissue samples was extracted with water and ethanol to 5g of tissue sample, 5 ml of water and solvent were added and ground well with mortar and pestle water and ethanol extract were centrifuged at 15000 rpm for 30 min and the supernatants were stored at -20^0 C until use.

Antibacterial activity of gastropod extracts

In vitro antibacterial activity was determined by agar well diffusion technique (Perez *et al*., 1990), 100 mg of lyophilized powder was dissolved in 1ml of water. This agar plates were incubated at 37 ˚C for 24 hours. Three replicates were formed for every bacterium (Cruickshank *et al*., 1975), inhibition zone was measured (mm). Antimicrobial antibiotics disc: Ciprofloxacin were used as antibacterial standards.

Gastropod crude extract was tested for inhibition of bacterial and fungal growth against human pathogenic bacteria. Microbial assay was carried out by disc diffusion technique followed by Kelman *et al.* Five species of pathogenic bacteria namely *Klepsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus , Enterococcus faecalis* were obtained from Muthaiya Research Laboratory, Thanjavur was used for screening the antibacterial activity of the gastropod extracts. Pathogenic bacterial strains were inoculated in sterile nutrient broth and incubated at 37 ^{0}C for 24 h. Pathogens were swabbed on the surface of the Muller Hinton agar plates and discs (Whatman No.1 filter paper with 3 mm diameter) impregnated with 50 µL of gastropod extracts placed on the surface. Control discs were with water and solvents to assess the effect of water and solvents on pathogens. The plates were incubated at 37 ^{0}C for 24 h and the antimicrobial activity was measured accordingly based on the inhibition zone around the disc impregnated with gastropod extract.

Fourier transform infrared spectroscopy (FTIR) spectral analysis

The samples of *Pila virens* (10mg) were mixed with 100 mg of dried potassium bromide (kbr) and compressed to prepare as a salt disc. The disc was then read spectro photometerically (Bio-Rad FTIR-40-model, USA).The frequencies of different components present in each sample were analyzed.

Results and Discussion

In recent years, great attention has been paid to study the bioactive of natural products because of their potential pharmacological utilization. Most homeopathic medicines are either from plant or animal origin (Bansemir *et al*., 2006). More than 1,100 antibiotic substances have been isolated from invertebrates. Among these, 50 have found widespread use in the prevention and treatment of bacterial diseases in animal and man (Gale and Kiser., 1967). In the present study a total of two crude extracts from the *Pila virens* was screened against five human pathogenic bacteria strains for antibacterial activities (Table:1). After the evaporation of the solvents the extracts were brown (water), yellow (Ethanol) in colour. These were used for further determination of antimicrobial activity. The inhibition zones of extracts against the specific test organisms were measured. The extract restricted the growth of pathogens on the media around the impregnated discs. Ethanol extract of *Pila virens* was found active against five species of pathogens. The inhibition zone ranged from 9 mm to 11 mm. The maximum inhibition zone was 11 mm against *Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecalis.* The minimum zone was 9mm in *Salmonella typhi* and 10mm in *Klepsiella pneumoniae.* Water extract of *Pila virens* zone ranged from 9mm to 10mm (Table: 2)(Fig:2). The maximum inhibition zone was 10mm in *Staphylococcus aureus, Enterococcus faecalis* and the minimum inhibition zone was 9mm in *Klepsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi.* The positive control (Ciprofloxacin) showed activities against to five pathogen bacterial strains. The inhibition zone ranged from 24 mm to 30 mm.

FTIR spectral analysis

The FTIR spectra of the samples of the 9 major peaks were at 2851.32, 1682.39,1488.96, 1337.99, 1117.63, 1036.13,876.16, 747.44 and 568.15 cm^{-1}, whereas the spectra of the sample of *Pila virens* showed all peaks with very close values at 3388.18, 2956.67, 1653.06, 1457.51, 1084.75, 989.53, 783.95, 620.82 and 420.56 cm^{-1}. (Fig:3)

The distinct antibacterial activity was observed against almost all the pathogenic bacteria. Ethanol extracts of *Pila virens* showed highest activity against *Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecalis.* Whereas, water extracts showed highest activity against *Staphylococcus aureus, Enterococcus faecalis.* Antibacterial activity of

gastropods against *Salmonella typhi* was reported by (Rajaganapathi, 1996). Studied the antibacterial activities in ethanol extracts of gastropod *Babylonia spirata* and *Turbo brunneus* and observed highest activity against *E.coli, K. pneumoniae, P.vulgaris* and *S.typhi* (Anand and Patterson Edward., 2001). The results may indicate the presence of different natural antibacterial substances in the molluscs tested, which gave inhibition zones on plates with different pH and test bacteria. It is almost impossible to detect the residues of antibiotics in molluscs because these substances are not used for the treatment of diseases of these organisms due to economical and environmental reasons (Radgers , 2009).

Many antimicrobial screening studies have shown that Gram-negative bacteria are more sensitive than Gram-positive bacteria (Tadesse *et al.*, 2008). Nevertheless in our screening study, water and ethanol extract were highly effective against both groups of bacteria, including several human pathogens, as an interesting activity has been discovered in these molluscs, the bioactive compound nature needs to be specified through more purification, steps and should lead to further studies relating to their antibacterial modes of action otherwise, a microbial origin of these active compounds cannot be ruled out. Commercial antibiotics are highly effective to kill the bacterial and fungal pathogens involved in common infection. Water, ethanol and methanol extracts of gastropods used in the present study showed significant antibacterial activity compare with other solvents extraction. FTIR analysis reveals the presence of bioactive compounds signals at different ranges. The research shows that the medicinal value of the gastropod *P.virens* muscle may be due to high quality of antibacterial compounds.

Conclusion

In traditional Indian medicine, especially Sidha medical preparations, the opercula of gastropods are used as an ingredient to compact different diseases. It is worthy to note that the product from natural source is good for health and to avoid side effects. Among these 50 have found widespread use in the prevention and treatment of bacterial disease in animal and man. The present study revealed that the species of *Pila virens* showed antimicrobial activities against the pathogenic microbial forms. So they possess potential pharmacological action.

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Table 1: Antibacterial (Zone inhibition in mm) of different extracts in Freshwater Gastropod (*Pila virens*)

Species	Positive control (Ciprofloxacin)	Different extracts of *P.virens*	
		Aqueous	Ethanol
Klepsiella pneumoniae	29 mm	9 mm	10 mm
Pseudomonas aeruginosa	30 mm	9 mm	11 mm
Salmonella typhi	26 mm	9 mm	9 mm
Staphylococcus aureus	24 mm	10 mm	11 mm
Enterococcus faecalis	27 mm	10 mm	11 mm

Table 2: Antibacterial activity of Aqueous & Ethanol extract from *P. virens* (Inhibition zone in hours)

Hours	*Klepsiella pneumoniae*		*Pseudomonas aeruginosa*		*Salmonella typhi*		*Staphylococcus aureus*		*Enterococcus faecalis*	
	Aqueous	Ethanol	Aqueous	Ethanol	Aqueous	Ethanol	Aqueous	Ethanol	Aqueous	Ethanol
0	3	3	3	3	3	3	3	3	3	3
4	3.5	4	4.5	5	4	4.5	5	6	4	5.5
8	4.5	6.8	5.5	6.5	4.5	5	6.5	7.5	6	7
12	6.5	7.5	7	8	6	6.5	8.5	8	7.5	8.5
16	7.5	8.5	8.5	9.5	7	7.5	9	9.5	8	9
20	8	9.5	9	10.5	8.5	8.5	9.5	10	9.5	10.5
24	9	10	9	11	9	9	10	11	10	11

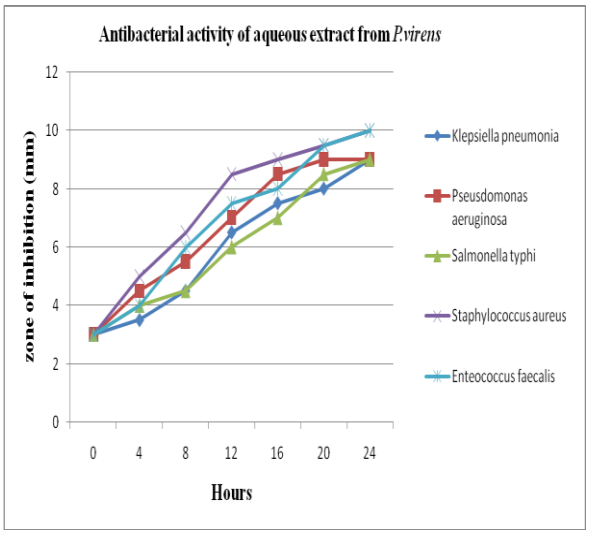

Fig. 1:

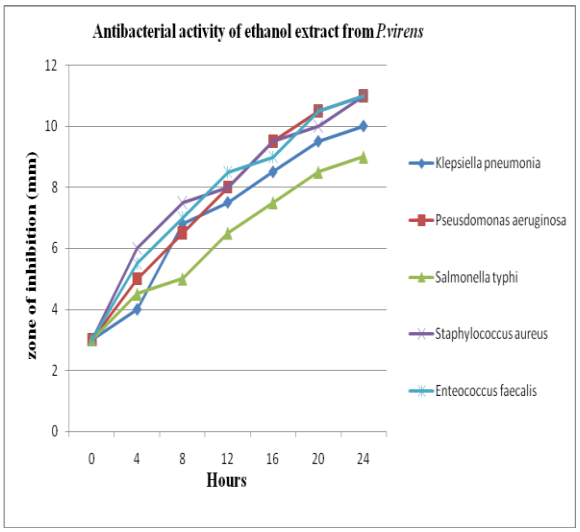

Fig. 2:

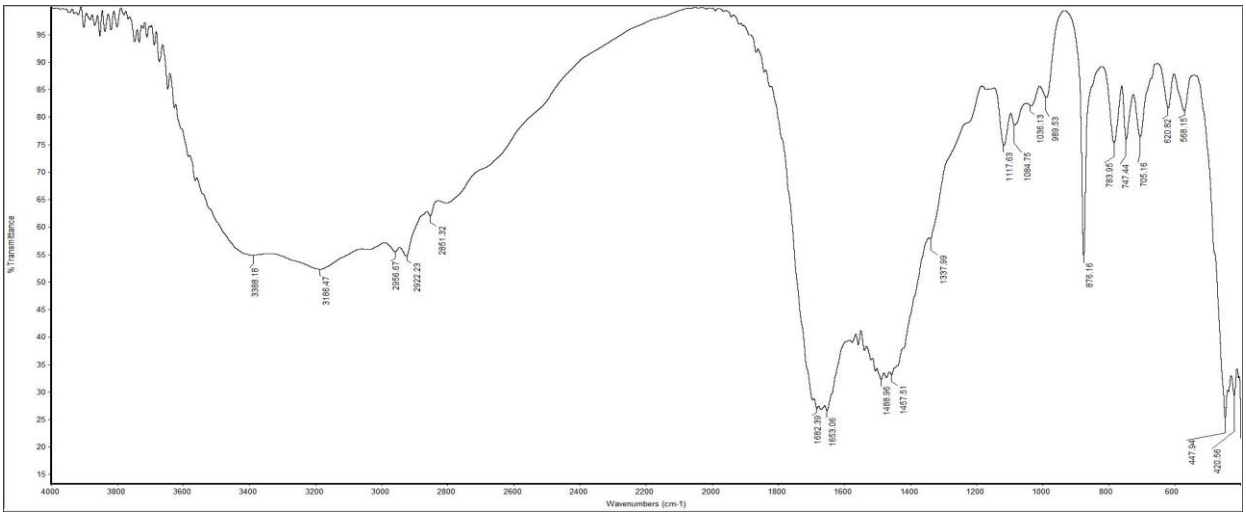

Fig. 3: The FTIR spectra of sample in *Pila virens*

How to cite this article
Gayathri M. and Sanjeevi S. Baskara (2014). Antipathogenic activity of freshwater gastropod *Pila virens* (Lamarck, 1822) from Lower Grand Anaicut Reservoir, Tamilnadu. *Int. J. Pharm. Life Sci.*, 5(10):3894-3898.
Source of Support: Nil; Conflict of Interest: None declared

Received: 25.09.14; Revised: 05.10.14; Accepted:08.10.14