Research Article [Santhiya & Sanjeevi, 5(10): Oct., 2014:3899-3902]
CODEN (USA): IJPLCP ISSN: 0976-7126

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

Antibacterial activity of freshwater Mussel *Parreysia corrugata* (Muller 1774) from Lower Anaicut Reservoir, India

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Abstract

In the present study aqueous, ethanol and methanol extracts of freshwater mussel *Parreysia corrugata* were screened for antibacterial activity. The extracts were obtained from the whole body tissue of the animals and tested against 5 pathogenic bacteria viz., *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Enterococcus faecalis* and *Staphylococcus aureus*. Ethanol extract of *P.corrugata* showed maximum antibacterial activity 11 mm against *P.aeruginosa* and the minimum activity 9 mm against *K. pneumoniae.* Methanol extract of *P.corrugata* showed Maximum inhibition 13mm against *P.aeruginosa* and the minimum inhibition zone of 8mm against *K. pneumoniae*. Aqueous extract of *P.corrugata* showed the maximum inhibition zone was 10mm against *S.aureus* and the minimum inhibition zone was 7 mm against *S.typhi*. Aqueous, Ethanol and Methanol extracts showed antibacterial activity against five human pathogen bacteria tested. Compare to water extracts Ethanol and Methanol extracts showed more activity against all pathogens. FTIR analysis revealed the presence of bioactive compounds signals at different ranges.

Key-Words: *Parreysia corrugata*, Antibacterial activity, AMPs, Pathogens

Introduction

Molluscs are far and wide dispersed all over the world namely slugs, whelks, clams, mussels, oysters, scallops, suits and octopus. Ocean includes a huge biodiversity of plants and animals which is predictable to be over 5, 00,000 species more than twice over of the land species (Charlet *et al.* 1996).

Many classes of molluscs exhibits bioactive compounds like antitumor, antibacterial and antiviral properties have been reported worldwide Patit *et al.*, (1987); (Kamiya *et al.*, (1989); Anand *et al.*, (2001); Rajaganapathi *et al.*, (2002). Among the mollucs some animal exhibited pharamacological activities or other properties which are useful in the biomedical area.

Freshwater mussels were widely distributed in Indian water bodies. Due to an alarming rise in the occurrence of antibiotic resistant bacterial strains, the identification of new antimicrobial compounds has become one of the frontier areas in biomedical research.

Aquatic (marine and freshwater) invertebrates are known to rely on innate immune mechanisms which include both interacting cellular and humoral components to protect against potential pathogen (Tincu and Taylor, 2004).

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Innate immune mechanism in freshwater invertebrates is known to protect these organisms against potential pathogens. Moreover, it has been well known that the innate immunity is triggered immediately after microbial infection to produce antimicrobial compounds including small antimicrobial peptides (AMP). In recent years, it has widely been recognized that AMPs are strong defensive weapons against bacteria and/or fungi, viruses, or parasites in multicellular organisms (Zasloff, 2002). Furthermore, AMPs are also known as major components of innate immune defence system in invertebrates (Seo *et al*, 2005). Considering the fact that the aquatic animals can survive in a hostile environment where they are surrounded by various pathogenic organisms, including human pathogens (Bouchriti and Goyal, 1992) and that they are potential sources for bioactive compounds, an attempt has been made in the present study to evaluate the antimicrobial activity in commonly occurring edible bivalve, such as *Parreysia corrugata*. In continuation with the same effort, an attempt was also made to assess and compare the efficacy of the extracts prepared using three different extraction procedures.Freshwater bivalves provide significant ecological benefits recognized as a source of food for

human beings and also for other domestic animals from all over world.

Material and Methods

Collection and extraction of samples

Live mussels *Parreysia corrugata* used in the present study were collected from Lower Anaicut Reservoir, India (Lat 11^0 15'N and long70^0 30' E). These bivalves were not collected during the summer months to avoid stress related to disease, elevated water temperature, hypoxia or gametogenesis. Bivalves were brought to the laboratory in water, washed, and de-shelled; tissue and mantle fluids were also collected. Bioactive compounds form thetissues sample 5ml of water and solvent were added and ground well with mortar and pestle water solvent extract were centrifuged at 15000 rpm for 30 min and the supernatants were stored at – 20^0 C until use.

Antibacterial activity of Bivalve Extract

Five species of pathogenic bacteria namely *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Enterococcus faecalis* and *Staphylococcus aureus* were obtained from the Muthaiah Research Labaratory, Thanjavur was used for screening the antibacterial activity of the Bivalve extracts. Pathogenic bacterial strains were inoculated at 37^0 C for 24 hrs. Pathogens wereswabbed on the surface of the Muller Hinton agar plates and the discs were (Whatman No. 1 Filter paper with 3mm diameter) impregnated with the 50µl of Bivalve extracts. The discs were placed on the surface of the plate. Control discs were placed with water and solvents to access the effect of water and solvent on pathogens. The plates were incubated at 37^0 C for 24 hours and the antibacterial activity was measured based on the inhibition zone around the disc impregnated with bivalve extract.

Fourier transform infrared spectroscopy (FTIR) spectral analysis

The samples of *P.corrugata* (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 the present study, a total 3 crude extracts from the *Parreysia corrugata* was screened against 5 human pathogenic bacterial strains for antibacterial activities. After evaporation of the solvents, the extracts were brown, light yellow and white in colour and were thick. These were used for further determination of antibacterial activity. The inhibition zones of extracts against the specific test organisms were measured. The extract restricted the growth of bacteria on the media around the impregnated discs. Ethanol extract of *P.corrugata* was found active against five species of bacteria's. The inhibition zone ranged from 9mm to 11 mm. The minimum inhibition zone was 9mm , against *K. pneumoniae.* The maximum inhibition zone was 11 mm against *P.aeruginosa*. Methanol extract of *P.corrugata* showed. Maximum inhibition 13mm against *P.aeruginosa* and the minimum inhibition zone of 8mm against *K. pneumoniae*. Aqueous extract of *P.corrugata* showed activities against the 4 bacterial strains. The maximum inhibition zone ranged from the 7 mm to 10mm. The maximum inhibition zone was 10mm against *S.aureus* and the minimum inhibition zone was 7 mm against *S.typhi*(Figure 1).

FTIR spectral analysis

The FTIR spectra of the samples of the 9 major peaks were at 2.853.50, 1558.44, 1540.72, 1508.15, 1457.46, 1248.85, 872.03, 667.42 and 483.06 cm^{-1}, whereas the spectra of the sample of *P.corrugata* showed all peaks with very close values at 3420.08, 2924.94, 1652.62, 1030.00, 462.80, 448.62 and 416.21cm^{-1}.

Most of the work carried out on antimicrobial compounds from marine bivalves deal with *M. edulis, M. galloprovicialis, G. demissa, C. verginica*and *C. gigas* (Mitta *et al*, 1999; Tincu and Taylor, 2004; Haug*et al*, 2004). In this study, an attempt has been made to screen freshwater bivalves, especially the commonly occurring edible ones. The source for majority of the AMPs reported has been from the hemocytes (Charlet*et al*, 1996; Mitta *et al*, 1999) epithelial tissues (Marshall and Arenas, 2003; Noriaki*et al*, 2003) and the tissues of gut and respiratory organs (Tincu and Taylor, 2004). Considering this an important aspect, the extracts were prepared using both mantle tissue and mantle fluid of the bivalves.Bivalve mussels live in the environment, in which up to 106 of different bacteria and up to 109 viruses per ml of water may exist (Tincu and Taylor., 2004), (Harris *et al.,* 2009),(Diaz ., 2010). As filter-feeding organisms, molluscs are exposed to high concentrations of bacteria, including pathogens. For this reason their immune defenses system must be based on non-specific, rapid cellular and humoral responses. Since 1980s, many different antimicrobial substances identified in molluscs were described and characterized (Hubert*et al.,* 1996), (Bulet*et al*., 2004), (Haug*et al*.,2004), (Dorringtone*t al.,* 2008).

Conclusion

Freshwatermussel *Parreysia corrugata* is widely distributed in freshwater bodies of Indian sub-continent. The animal is reported to be medicinally important and used by aboriginal people to control

blood pressure. It is also used in cement, lime,button, toys and cosmetic industries. In certain parts of the country, the animal is consumed as food by poor people. Recently, successful pearl production has been reported using this species in the state of Orissa.

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Table 1: Antimicrobial activity (zone of inhibition in mm) of different extracts of freshwater mussel (*P. corrugata*)

Test organism	Different extracts of *Parreysia corrugata*		
	Aqueous	Ethanol	Methanol
K. pneumoniae	9 mm	9 mm	8 mm
P.aeruginosa	8 mm	11 mm	13 mm
S.typhi	7 mm	10 mm	12 mm
S.aureus	10 mm	10 mm	9 mm
E.faecalis	-	10 mm	11 mm

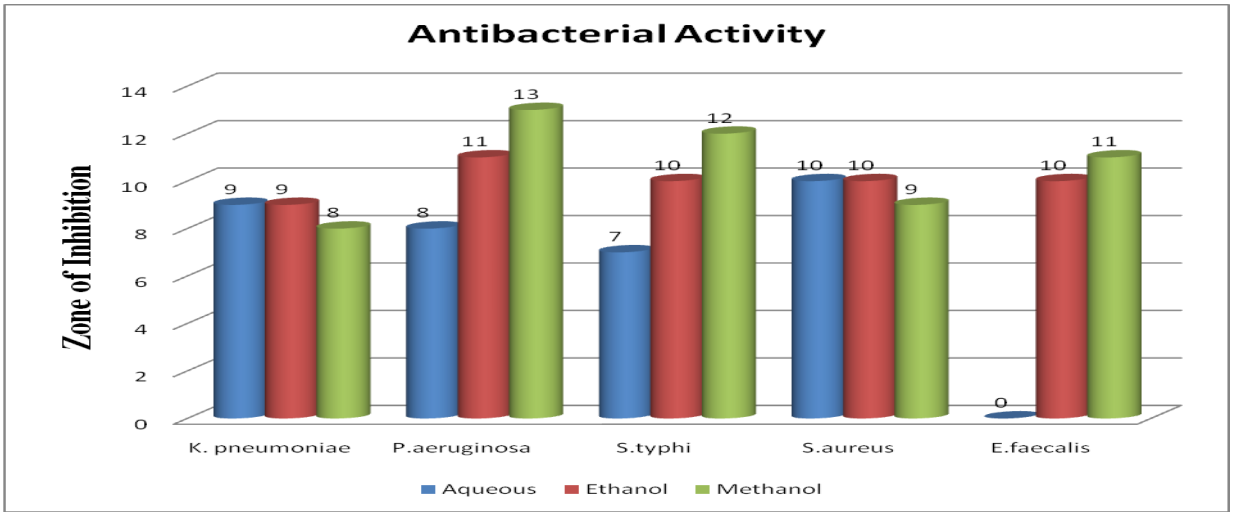

Fig. 1: Diameter of inhibition zone of freshwater mussel *Parreysia corrugata* against each test microorganisms

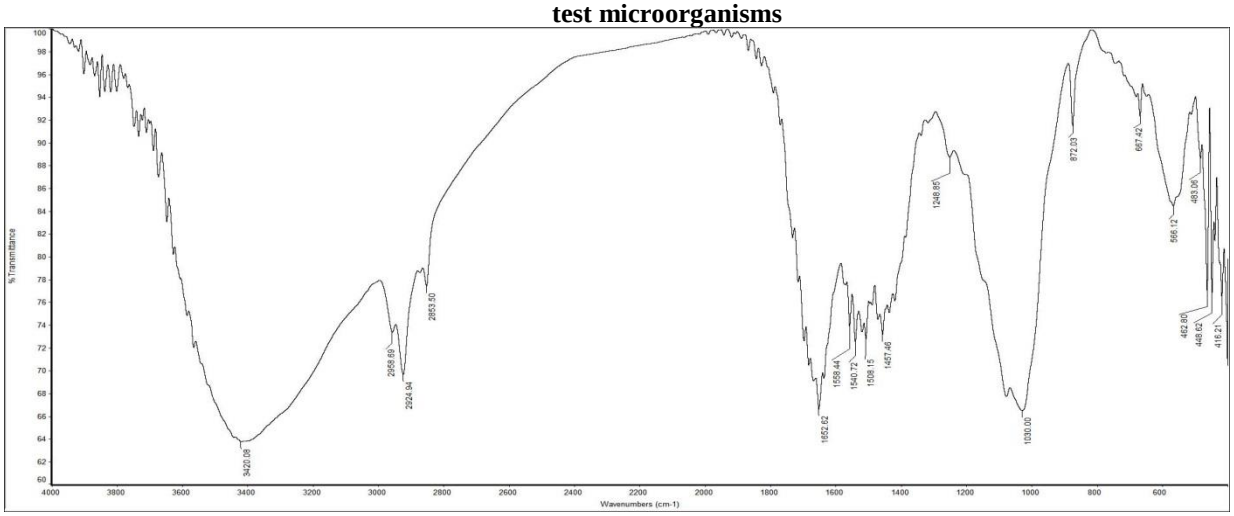

Fig. 2: FTIR spectra of sample in *Parreysia corrugata*

How to cite this article

Santhiya N. and Sanjeevi S. Baskara (2014). Antibacterial activity of freshwater Mussel *Parreysia corrugata* (Muller 1774) from Lower Anaicut Reservoir, India. *Int. J. Pharm. Life Sci.*, 5(10):3899-3902.

Source of Support: Nil; Conflict of Interest: None declared

Received: 25.09.14; Revised: 05.10.14; Accepted:08.10.14