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Antibacterial Activity of Freshwater Bivalve *Lamellidens marginalis* (Lamarck, 1819) From Lower Anaicut Reservoir, India

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ABSTRACT

Aquatic invertebrates are known to rely on innate immune mechanisms which include both interacting cellular and humoral components to protect against potential pathogen .In the present study aqueous, ethanol and chloroform extracts of freshwater mussel Lamellidens maginalis were screened for antibacterial activity. The extracts were obtained from the whole body tissue of the animals and tested against 5 pathogenic bacteria viz., Streptococcus pyrogenes, Staphylococcus aureus, Serratia marganii, Klebsiella oxytoca and Lactobacillus vulgaris. The inhibition zone ranged from 7mm to 13 mm. The minimum inhibition zone was 7mm, against K.oxytoca. The maximum inhibition zone was 13 mm against L.vulgaris. Aqueous extract of L. maginalis showed activities against the 5 bacterial strains. The maximum inhibition zone ranged from the 9 mm to 11mm. This data suggests that L.marginalis is the potential candidates for the isolation and purification of potent antibiotics. Moreover, the extraction of the antimicrobial compound appeared to be dependent on the extraction procedure and the nature of the solvents used for extraction.

Keywords: *Lamellidens marginalis. Mollusc, Antibacterial activity, Pathogen, antimicrobial compound*

INTRODUCTION

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[10]. 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[11]. Furthermore, AMPs are also known as major components of innate immune defense system in invertebrates[8]. Considering the fact that the aquatic animals can survive in a hostile environment where they are surrounded by various pathogenic organisms, including human pathogens[1] 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 *Lamellidens maginalis*.

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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.

MATERIALS AND METHODS

Live *Lamellidens maginalis* bivalves 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 the tissues 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

5 species of pathogenic bacteria namely *Streptococcus pyrogenes, Staphylococcus aureus, Serratia marganii, Klebsiella oxytoca and Lactobacillus vulgaris* 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 were swabbed 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 disc 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.

RESULTS

Freshwater mussels are filter feeder animals that up-take and concentrate the contaminants and faecal coliform bacteria from its surrounding waters and thus act as indicators of health of its environment. Attempts have been carried out outside India to use this important component of aquatic ecosystem to monitor its health and pollution level. However, in India there is dearth of information available on such studies. Hence, an attempt was made to carry out analysis of the tissues of freshwater mussel, *Lamellidens maginalis* and its surrounding water to confirm these earlier. Findings in other freshwater bivalves.

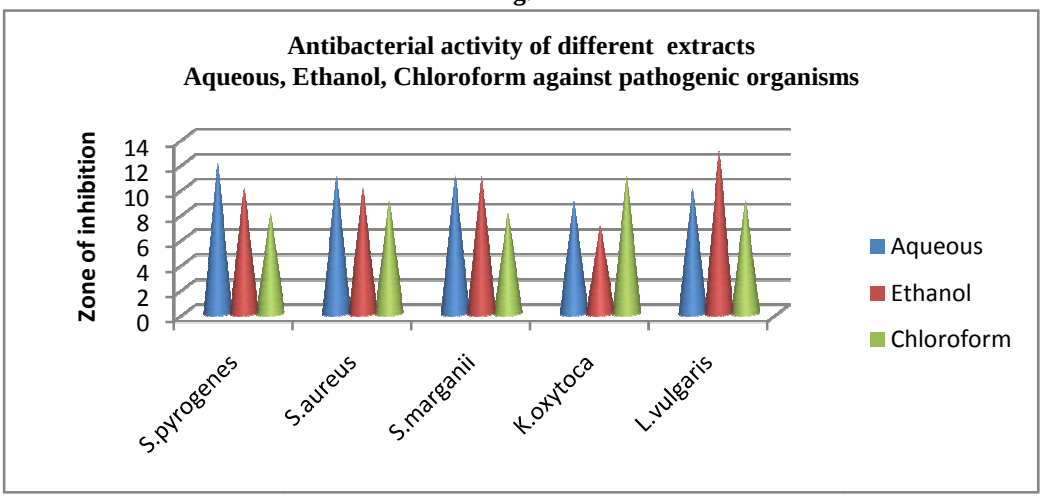
In the present study, a total 3 crude extracts from the *Lamellidens maginalis* was screened against 5 human pathogenic bacterial strains for antibacterial activities. After evaporation of the solvents, the extracts were brown, light yellow and white in colour and were thick. These were used for further 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 *L. maginalis* was found active against five species of bacteria.

The inhibition zone ranged from 7 mm to 13 mm. The minimum inhibition zone was 7 mm, against *K. oxytoca.* The maximum inhibition zone was 13 mm against *L.vulgaris.* Aqueous extract of *L. maginalis* showed activities against the 5 bacterial strains. The maximum inhibition zone ranged from the 9 mm to 11mm. This data suggests that *L.marginalis* is the potential candidates for the isolation and purification of potent antibiotics. Moreover, the extraction of the antimicrobial compound (s) appeared to be dependent on the extraction procedure and the nature of the solvents used for extraction.

Table 1

Test organism	Different extracts of *Lamellidens marginalis*		
	Aqueous	**Ethanol**	**Chloroform**
S.pyrogenes	12	10	8
S.aureus	11	10	9
S.marganii	11	11	8
K.oxytoca	9	7	11
L.vulgaris	10	13	9

Fig. 1



DISCUSSION

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*[10]. 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[2] epithelial tissues and the tissues of gut and respiratory organs[10]. Considering this an important aspect, the extracts were prepared using both mantle tissue and mantle fluid of the bivalves.

Presuming that the antimicrobial compounds were either protein or peptide in nature, a combination of 'soft techniques' was selected for the preparation of crude methanol (homogenization with chilled methanol + filtration) and chloroform extracts (homogenization with chloroform + protease inhibitor + centrifugation) to ensure that the functionality and/or the biological activity of the analyses remained intact.

For this, methanol was selected as a suitable solvent as it gave good extraction efficiency. Most of the low molecular weight proteins/peptides (stable at room temperature) were extracted from it and had an added advantage of allowing rapid sample concentration through evaporation. The other solvent/homogenization medium is chloroform as most of the active high molecular weight proteins and peptides are known to be extracted in it. Protease inhibitor simultaneously deactivated proteolytic enzymes in the tissue which would otherwise cause rapid degradation of the proteins/peptides[3]. The results of antimicrobial assay in the present study indicated that these extracts showed high antimicrobial activity against the tested pathogens, indicating that these procedures are capable of extracting the antimicrobial compound, with relatively higher activity, without degrading the nature of the compound.

The acetone extraction procedure employed in the present study was very harsh as all the conditions (e.g. highly acidic pH, 100°C temperature) were totally different from the physiological conditions of the biological matrices. Enzyme protosubtilin hydrolyzed high-molecular animal proteins to short peptides and a mixture of free amino acids. The crude extract has been reported to possess short peptides, free amino acids (conjugated with metals like Cu, Zn etc) and minerals. They were also reported to possess low fat and salt contents[2].The antimicrobial activity of the acetone extract might be either due to short peptides, amino acids conjugated with metal ions or both or it could also be due to the generation of artifacts during the extraction process. The standard disc diffusion method is a sensitive and highly accepted method used for the detection of antimicrobial activity, but many feel that it is a qualitative method and should not be used to quantify the activity[7]. Therefore, the results were quantified using the liquid growth inhibition assay. These results largely confirmed the findings of disc diffusion assay and helped in calculating the inhibition percentage of culture growth caused by a particular extract towards the pathogenic organism. This assay was carried out for 30 hrs to compare the time when the extracts started inhibiting the growth of the organism and when it lost the activity. It was observed that most of the

crude extracts were able to induce inhibition for ±10 hrs and showed antimicrobial activity with a purified antibiotic like Gentamycin.

The results presented in this paper are the first stage of a bioassay-based baseline survey to achieve the isolation, purification, structure elucidation and biological testing of the active compounds from the potent marine bivalves. This data suggests that *L. marginalis* is the potential candidates for the isolation and purification of potent antibiotics. Moreover, the extraction of the antimicrobial compounds appeared to be dependent on the extraction procedure and the nature of the solvents used for extraction.

In a previous study, a similar fractionation procedure was used to study antibacterial and fungal activities of seven ascidians, six sponges and one soft alcyonid coral. The highest antifungal and antibacterial activities were detected in the 40% and 80% SPE (Solis Phase Extraction) fractions. The 10% SPE-fractions assayed were generally active but less potent then the other fractions against bacteria and fungi. The screened species and the extraction procedure may explain the results. The active compounds detected from cnidaria and tunicate and those detected in this study from molluscs may differ at the structural level.

CONCLUSION

Bivalve *Lamellidens maginalis* 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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