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Antimicrobial activities of the tissue extracts of *Babylonia spirata* Linnaeus, 1758 (Mollusca: Gastropoda) from Thazhanguda, southeast coast of India

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ABSTRACT

Objective: To investigate the antimicrobial activity of the tissue extracts of *Babylonia spirata* (*B. spirata*) against nine bacterial and three fungal pathogens. **Methods:** Crude extract of gastropod was tested for inhibition of bacterial and fungal growth. Antibacterial assay was carried out by disc diffusion method and *in vitro* antifungal activity was determined against Czapex Dox agar. The antimicrobial activity was measured accordingly based on the inhibition zone around the disc impregnated with gastropod extract. Molecular size of muscle protein was determined using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). And fourier transform infrared spectroscopy (FTIR) spectro photometry analysis was also studied. **Results:** The maximum inhibition zone (12 mm) was observed against *Pseudomonas aeruginosa* in the crude ethanol extract of *B. spirata* and the minimum inhibition zone (2 mm) was noticed against *Staphylococcus aureus* in the crude methanol extract of *B. spirata*. Water extract of *B. spirata* showed the highest activity against *Vibrio parahaemolyticus*, *Staphylococcus aureus* and *Candida albicans*. Ethanol, acetone, methanol, chloroform and water extracts showed antimicrobial activity against almost all the bacteria and fungus. Compared with water extracts, ethanol and methanol extracts showed higher activity against all pathogens. The molecular weight of protein of the gastropod sample ranged from 2-110 kDa on SDS-PAGE. FTIR analysis revealed the presence of bioactive compounds signals at different ranges. **Conclusions:** The research shows that the great medicinal value of the gastropod muscle of *B. spirata* may be due to high quality of antimicrobial compounds.

1. Introduction

The marine environment is a huge source to discover bioactive natural products. A wide variety of bioactive substances are being isolated and characterized from the food that is derived from the marine environment, several with great promise for the treatment of human and fish disease. For the past two decades, pharmaceutical industry has been relatively successful in overcoming problems due to single resistant determinants. However, the advent of multiple resistant mechanism has limited the use of many major classes of antimicrobial compounds. The demand for effective and non-toxic antibacterial therapeutics has become even greater with the increased incidence of bacterial infections. There is a vital interest in discovering new antimicrobial compounds with fewer environmental and toxicological risks and no resistance developed by the pathogens[1]. In marine invertebrates so far approximately 7 000 marine natural products have been reported, 33% from sponges, 18% from coelenterates (sea whips, sea fans and soft corals), and 24% from representatives of other invertebrate phyla such as ascidians (also called tunicates), opisthobranch mollusks (nudibranchs, sea hares, *etc*), echinoderms (starfish, sea cucumbers, *etc*) and bryozoans (moss animals). In India, till today, 5 070 species of Mollusca have been recorded, among which 3 370 species are from marine environment[2], while rest from the fresh water and terrestrial environment.

Molluscs are widely distributed throughout the world and have many representatives such as slugs, whelks, clams, mussels, oysters, scallops, squids and octopods in the marine and estuarine ecosystem. Many classes of bioactive compounds exhibiting anti-tumor, anti-leukemic,

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antibacterial and antiviral activities have been reported world wide[3-5]. Among the molluscs some have pronounced pharmacological activities or other properties which are useful in the biomedical arena. It is surprising to find that some of the pharmacological activities are attributed to the presence of polysaccharides particularly sulphated muco polysaccharide. Antimicrobial peptides are important in the first line of the host defense system of many animal species[6]. 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 is largely explored in India. Therefore, the aim of the present study was to evaluate the antimicrobial activity of the tissue extracts of gastropod *Babylonia spirata* (*B. spirata*) against different pathogenic bacterial and fungal strains.

2. Materials and methods

2.1. Collection and extraction of samples

Live specimens of *B. spirata* (Family: Buccinidae) were collected from Thazhanguda coastal waters (latitude: 11^0 45′ 0N; longitude: 79^0 45′ 0E) Cuddalore, southeast coast of India. They were immediately brought to the laboratory and their soft bodies were removed by breaking the shells. The whole body muscle of the sample (50 g) was cut into small pieces and the tissue sample was used for extraction using different solvents such as ethanol, acetone, methanol, chloroform and water. The extracts were cold steeped over night at −18 ℃ and filtered with Whatman No. 1 filter paper. The filtrate was poured in previously weighted Petri plate and evaporated to dryness in rotary evaporator[7,8]. The dried crude extracts were used for antimicrobial assay against human pathogens [*Pseudomonas aeruginosa* (*P. aeruginosa*), *Vibrio cholera* (*V. cholera*), *Vibrio parahaemolyticus* (*V. parahaemolyticus*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*), *Streptococcus pneumoniae* (*S. pneumoniae*), *Salmonella typhi* (*S. typhi*), *Proteus mirabilis* (*P. mirabilis*), *Aspergillus flavus* (*A. flavus*), *Candida albicans* (*C. albicans*) and *Mucor* sp.]. All the pathogenic bacterial and fungal strains were obtained from Raja Muthiah Medical College, Annamalai University.

2.2. Antimicrobial assay

Gastropod crude extract was tested for inhibition of bacterial and fungal growth against human pathogenic bacteria and fungi. Microbial assay was carried out by disc diffusion technique followed by Kelman *et al*[9]. Pathogenic bacterial strains were inoculated in sterile nutrient broth and incubated at 37 ℃ for 24 h. Pathogens were swabbed on the surface of the Muller Hinton agar plates and discs (Whatman No.1 filter paper with 9 mm diameter) impregnated with 50 μL of gastropod extracts placed on the surface. *In vitro* antifungal activity of gastropod crude extract was determined against Czapex Dox agar. Inoculums of 24 h old culture of *A. flavus* well drained spores were distributed uniformly on the surface of the agar plates with the help of sterile cotton swab. *Mucor* sp. and *C. albicans* fungal strains were inoculated by taking a piece of fungal colony using a sterile cotton swab and gently swabbed on the surface of the medium. Control discs were with water and solvents to assess the effect of water and solvents on pathogens. The plates were incubated at 37 ℃ for 24 h and the antimicrobial activity was measured accordingly based on the inhibition zone around the disc impregnated with gastropod extract.

2.3. Molecular size of muscle protein by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

Molecular size of muscle protein was determined using SDS-PAGE gel following the procedure by Sambrro *et al*[10]. Glass plates were assembled and 20 mL of 15% resolving gel was prepared and poured immediately to the notch plate. It was over laid with butanol. After polymerization was completed, it was poured off and the top layer was washed with deionized water. Then it was over laid with 8 mL of stock gel. Approximately 1 mL of 1% SDS gel loading buffer and sample were taken and it was heated at 100 ℃ for 3 min. Then it was assembly fixed in electrophoresis apparatus. 15 μL of samples with different molecular weight markers (6.5-97.4 kDa) were loaded, respectively in the well, run in the gel and stained with coomassie brilliant blue.

2.4. Fourier transform infrared spectroscopy (FTIR) spectral analysis

The lyophilized samples of *B. spirata* (10 mg) 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.

3. Results

3.1. Antimicrobial activity

Totally five crude extracts from one species of gastropod *B. spirata* were screened against nine human pathogenic bacteria and three fungal pathogens for testing their antimicrobial activities. The inhibition zones of ethanol, acetone, methanol, and chloroform extracts as compared with water extract against the specific test organisms were given in Figure 1. The maximum inhibition zone (12 mm) was

observed against *P. aeruginosa* in the crude ethanol extract of *B. spirata* and the minimum inhibition zone (2 mm) was noticed against *S. aureus*. As for fungi only *A. flavus* and *C. albicans* showed more activity on ethanol, acetone crude extract. One fungal pathogen showed negative results on ethanol crude extract. The acetone and methanol extracts were able to produce a zone of 6 mm against *S. aureus*, *C. albicans*, *V. cholera*, *K. pneumoniae* and *P. mirabilis*. However, only slight activity was shown by the crude extract of chloroform (Figure 1).

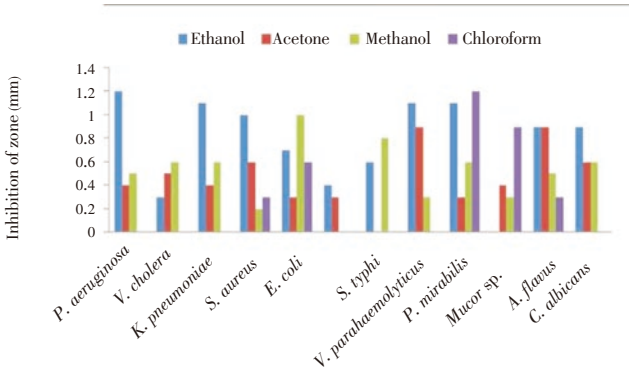

Figure 1. Diameter of inhibition zone of molluscs *B. spirata* against each test microorganism.

3.2. SDS-PAGE

Crude protein sample of *B. spirata* yielded 6 bands ranging from 2.0-110.0 kDa with well defined. The bands were at 110.0, 97.4, 45.5, 20.0, 4.5 and 2.0 kDa, respectively. Gastropod sample was compared with the standard protein molecular weight marker (6.5-97.4 kDa) (Banglore Genei, India) (Figure 2).

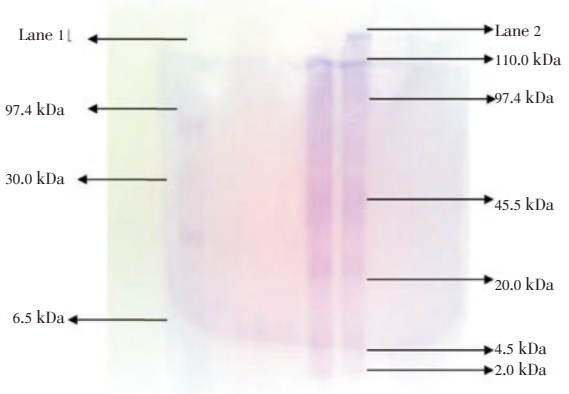

Figure 2. Molecular size of *B. spirata* muscle protein determined by SDS-PAGE.
Lane 1: Standard protein molecular weight marker; Lane 2: Crude protein profiles in *B. spirata*.

3.3. FTIR spectral analysis

The FTIR spectra of the lyophilized sample of the 8 major peaks were at 2 860.27, 1 484.93, 1 342.47, 1 218.14, 1 152.18, 933.63, 899.25 and 860.39 cm^{-1}, whereas the spectra of the sample of *B. spirata* showed all peaks with very close values at 3 388.75, 2 958.90, 2 925.32, 1 643.07, 1 539.73, 1 402.05, 673.97, 615.76, 536.99 and 465.75 cm^{-1} (Figure 3).

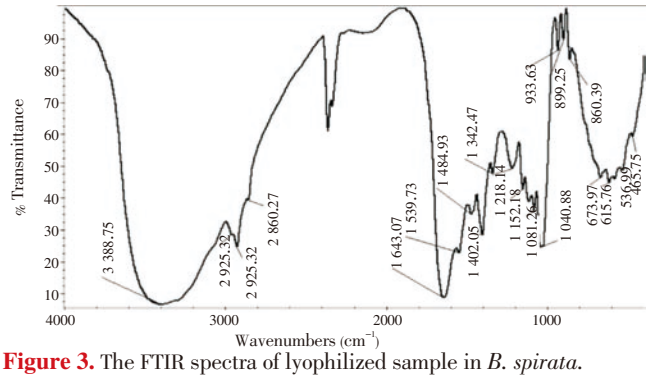

Figure 3. The FTIR spectra of lyophilized sample in *B. spirata*.

4. Discussion

There is a 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 extracted from higher plants. In traditional Indian medicine, especially Sidha medical preparations, the opercula of gastropods are used as an ingredient to combat different diseases. *B. spirata* are organisms of muddy bottom of shallow waters.

In the present study, a pronounced antimicrobial activity has been observed against some bacterial and fungal strains. The ethanol, acetone, methanol, chloroform and water crude extracts of *B. spirata* show activity against both bacterial and fungal strains. In antimicrobial activity the maximum zone of inhibition was recorded in *P. aeruginosa* strain and minimum zone inhibition was observed in *S. pneumonia* strain. The maximum antifungal activity was observed against *A. flavus* and minimum activity was recorded in *Mucor* sp. Similar result was reported in the antibacterial activities of ethanol extracts of gastropods *B. spirata* and *Turbo brunneus* showed the maximum activity against *E. coli*, *K. pneumoniae*, *P. vulgaris* and *S. typhi*[4]. The maximum antibacterial activity against *S. aureus* and *E. coli* by *Trochus radiates* was also reported[11]. These investigations support the present findings of the antimicrobial activity of muscle extraction of *B. spirata*. The antimicrobial activity was reported in four bivalves against few pathogens and found that extracts showed significant activity against *Bacillus subtillus*[12]. This study corroborates the results of the present investigation. The antimicrobial activity from the gill extraction of *Perna viridis* (Linnaeus, 1758)[13], antimicrobial activities of bivalve mollusk *Meretrix meretrix* (Linnaeus, 1758) and *Meretrix casta* (Gemelin, 1791)[14], antibacterial activities of green mussel (*Perna viridis*) and edible oyster (*Crassostrea madrasensis*)[15] were reported. It is also reported that the

acetone extract of the winged oyster *Pteria chinensis* was found to have a broad spectral activity inhibiting all the fish pathogenic strains tested and the extract of chloroform inhibited eight pathogens. These also support the present study on antimicrobial activity of gastropod extracts[1]. Difference in the antibacterial activity found in gastropod extract may depend on extracting capacity of the solvents and the compounds extracted.

The first attempt to locate antimicrobial activity in marine organisms was initiated around 1950′s[16]. Since this time, a large number of marine organisms from a wide range of phyla have been screened for antimicrobial activity[17]. Many of these organisms have antimicrobial properties, although most of the antibacterial agents that have been isolated from marine sources have not been active enough to complete with classicical antimicrobial activity against microorganisms[18]. The presence of antimicrobial activity in mollusca has been reported from the mucus of the giant snail *Achantina fulica*[19,20]. The methanol extract of *Hemifusus pugilinus* possessed the highest activity against *E. coli* and the lowest activity was observed against *Klebsiella oxytoca*. The methanol extracts of *Anadara granosa* showed the highest activity against *E. coli* and the lowest activity against *S. typhi*[21]. The maximum antibacterial inhibition zone was exhibited from acetone crude extract of *Trochus tentorium* against human pathogen *S. pneumonia*[22]. The flesh of *Meretrix meretrix* was used widely in India and China as a fisher folk medicine to treat several liver diseases like jaundice, hepatitis-A&B[23]. Likewise the steroid extract of *Meretrix meretrix* can inhibit the cell growth and induction of G1-phase cell cycle arrested in hepatoma cells[24]. Two edible bivalve species of *Perna viridis* and *Meretrix casta* showed the antifungal activities[25-39]. Gonzalez *et al*[40] reported the mantle tissues of the oyster *Crassostrea gigas*. A polyproline-type AMP (47 residues) isolated from the Chilean scllop (*Argopecten purpuratus*) showed antifungal activity against *Fusarium oxisporum* and *Saprolegnia parasitic*[41]. The methanol extract of *Sepia officinalis* showed the maximum inhibition zone against *E. coli* and *Lactobacillus vulgaris* and the minimum inhibition zone was recorded against *Salmonella paratyphi*[42]. The antibacterial and antifungal activities of various concentrations of the polysaccharides extracted from the cuttle bone of *Sepia aculeate* and *Sepia brevimana* were reported by Shanmugan *et al*[43]. The antibacterial activity of crude extracts of *Murex virgineus* exhibited the zone of inhibition ranged between 2 mm and 10 mm. Methanolic extract of *Murex virgineus* was estimated significant effective antifungal activity against all the tested strains[44]. Commercial antibiotics are highly effective to kill the bacterial and fungal pathogens involved in common infection. Water, ethanol, acetone, methanol and chloroform extracts of gastropod used in the present study showed significant antimicrobial activity compared with others solvents. It is worthy to note that the product from natural source is good for health and devoid of side effects. In the present investigation, muscle extraction that showed antimicrobial activity was subjected to SDS-PAGE to estimate the number and molecular weight of proteins present. After electrophoresis clear bands were detected in the gel which represented molecular weight of proteins ranging from 2 to 110 kDa. FTIR analysis reveals the presence of bioactive compounds signals at different ranges. The research shows that the medicinal value of the gastropod *B. spirata* muscle may be due to high quality of antimicrobial compounds.

The present study revealed that the species of *B. spirata* showed antimicrobial activities against the pathogenic microbial forms. So they possess potential pharmacological action. However, some novel and uncharacterized mechanisms of action that might ultimately benefit the ongoing global search for clinically useful antimicrobial agents need to be explored to explain the characteristic of antimicrobial activity of *B. spirata*.

Conflict of interest statement

We declare that we have no conflict of interest

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