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Bioactive potential of some intertidal molluscs collected from Mumbai coast,West coast of India

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ABSTRACT

Objective: To identify the bioactive potential of crude methanolic extracts of five gastropods and one bivalve collected from the west coast of India (Mumbai coast). **Methods:** Disc-diffusion assay and micro-dilution technique were used to test the bioactive potential of these molluscs against six pathogenic bacteria. **Results:** The crude methanolic extracts of all the molluscs showed significant activity against one or more bacteria tested. Minimum inhibitory concentration of crude methanolic extracts was found in the range of 0.10—0.35 mg/mL against human pathogenic bacterial strains. *Hemifusus pugilinus* and *Nerita sp*. extracts were most effective showing activity against all bacterial strains. *Hemifusus pugilinus* extract exhibited good activity against *Vibrio cholerae* contrary to earlier reports. Methanolic extract of *Gafrarium divaricatum* demonstrated considerable activity against both *Escherichia coli* and *Streptococcus pyogenes*. *Trochus radiatus* extract was inactive against all the bacteria tested except *Escherichia coli*. *Euchelus asper* extract was active against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. **Conclusions:** All the molluscs screened exhibited potential activity against one or more bacterial strain. Further work is in progress to isolate the active constituents of the extracts.

1. Introduction

The diversity of the ocean has been the source of unique chemical compounds, which holds tremendous pharmaceutical potential. In the period from 1969 to 1999 about 300 patents on natural products derived from marine organisms have been issued[1]. Most of these studies have restricted themselves to sponges and other soft-bodied organisms for the search of bioactive compounds. However, the vast variety of molluscs in the marine environment provides an enormous resource for natural products research[2].

There have been a few studies specifically dedicated to natural products isolated from marine molluscs[2,3]. The majority of research on natural products from the phylum Mollusca has been focused on primarily soft-bodied or shell-less molluscs, particularly nudibranchs and opisthobranchs[4,5]. However some studies have also been reported biological activity from shelled molluscs[6,7]; contrary to the general assumption that presence of a shell provides all the required protection from predation[5]. Work has also been done on the antimicrobial activity of egg masses of the molluscs[8]. However research investigations on the antimicrobial potential of the marine molluscs commonly found on Indian coast are rare.

The intertidal area off the coast of Mumbai is highly polluted due to continuous exposure to domestic sewage. Yet it boasts of a high diversity of molluscs, especially gastropods and sponges. Therefore, it is rational to search for novel antimicrobial agents in this marine environment, as these benthic organisms are living in a soup of microorganisms[9].

During present investigation the methanolic extracts of five

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gastropods *viz. Nerita* sp., *Euchelus asper* (Gmelin), *Hemifusus pugilinus* (Born), *Trochus radiatus* (Gmelin) and *Bursa tuberculata* (Brodrip) and one bivalve *Gafrarium divaricatum* (Gmelin) were screened for bioactivity against clinical isolates of six bacteria.

2. Materials and methods

2.1. Sampling

All the samples were collected manually from the Marine Drive rocky shore at low tide. The samples were brought to the laboratory and immediately frozen at −20 ℃. The shell was cracked open using a tight grip plier; whole body of all the animals was removed and subsequently washed with autoclaved distilled water to remove any attached debris. The opercula of all the gastropods were detached from their respective body and discarded. The reference specimen of each species was deposited in the Department of Zoology, Institute of Science.

2.2. Extract preparation

The whole body of each sample was cut into pieces, homogenised in a blender with methanol and kept on shaker for 24 h. The supernatant was then filtered and concentrated under vacuum on a Rotary Evaporator at low temperature and reduced pressure to get crude methanolic extract.

2.3. Antibacterial assay

Antibacterial activity was tested against six bacterial strains viz. *Escherichia coli*, *Pseudomonas aeruginosa*, Streptococcus pyogenes, *Klebsiella pneumoniae*, Bacillus subtilis and Vibrio cholera using standard agar disc-diffusion assays [10]. Each microorganism was inoculated on the surface of a nutrient agar plate at a concentration of 1.2×10^8–1.5×10^8 CFU/mL using a sterile glass spreader. Previously sterilized Whatman No. 1 filter paper discs (6 mm in diameter) were impregnated with 0.5 mg/mL solution of each crude extract and were placed on the surface of inoculated plate. Each plate also had a Streptomycin disc (0.1 mg/mL) as a positive control and methanol disc as negative control. The plates were incubated at 37 ℃ for 24 h. All the assays were carried out in triplicates. The bioactivity of the extracts was measured by calculating the diameter (mm) of the growth inhibition halos. Zones of growth inhibition greater than 7 mm were considered susceptible to crude extracts[11].

2.4. Determination of MIC

Minimum Inhibitory Concentration (MIC) of crude extract was determined for serially diluted concentrations (0.05–0.5 mg/mL) of crude methanolic extracts against each individual human pathogenic bacterial strain. Previously described disc diffusion assay was used to calculate the MIC[12]. Disc of Streptomycin of concentration ranging from 0.001–0.1 mg/mL respectively were used as a positive control and methanol was used as negative control. MIC of Streptomycin against each bacterial strain was also calculated by using the disc diffusion assay as described above.

3. Results

The results of the *in vitro* antibacterial assay of the marine organisms are summarized in Table 1. Distinct antibacterial activity was observed against all the pathogenic bacteria. The extracts of *Nerita* sp. and *H. pugilinus* exhibited broad spectrum activity against all the microorganisms tested. *Nerita* sp. showed weak activity (7–10 mm) against all the tested bacteria while *H. pugilinus* exhibited good activity (10–15 mm) against *V. cholerae*. *T. radiatus* extract was inactive towards all the bacteria tested except E. coli against which it was weakly active. Crude extract of E. asper was weakly active against *K. pneumoniae* and *P. aeruginosa*. The bivalve *G. divaricatum* extract was weakly active against *K. pneumoniae* and *P. aeruginosa* but demonstrated good activity against *E. coli* and *S. pyogenes*. *B. tuberculata* demonstrated weak activity against *E. coli*, *K. pneumoniae* and *P. aeruginosa*.

Table 2 shows the MICs of crude methanolic extracts and Streptomycin concentrations against tested bacteria. *H. pugilinus* and *G. divaricatum* extracts demonstrated lowest MIC value of 0.1 mg/mL against *V. cholerae* and *E. coli* strains respectively. Highest MIC value of 0.35 mg/mL was shown by *Nerita* sp. and *T. radiatus* extract against *S. pyogenes* and *E. coli* strains respectively. *K. pneumoniae* and *P. aeruginosa* were inhibited significantly by all extracts except *T. radiatus* whereas *E. coli* did not show any response to *E. asper extract*. For each of the six bacterial strains, the MIC of Streptomycin was also estimated. It was found to be lowest (0.002 mg/mL) against *K. pneumoniae* and highest (0.06 mg/mL) against *P. aeruginosa*. Thus, *P. aeruginosa* was the most resistant strain followed by *V. cholerae* (0.005 mg/mL). *E. coli*, *B. subtilis* and *S. pyogenes* (0.003 mg/mL) were equally resistant while *K. pneumoniae* (0.002 mg/mL) strain was the least resistant strain used in the study.

Figure 1 compares the inhibition zones of different molluscs against each bacterial strain. It can be observed that crude methanolic extract of *Nerita* sp. shows maximum activity against pathogenic bacteria *B. subtilis* and *K. pneumoniae* compared to other strains. Extracts of *E. asper*, *B. tuberculata*, *H. pugilinus* and *G. divaricatum* show equally high activity against *P. aeruginosa*, *V. cholerae*, *B. subtilis* and *S. pyogenes* were found to be resistant against all the extracts except those of *Nerita* sp. and *H. pugilinus*. Against *E. coli* the extract of *G. divaricatum* exhibited maximum activity while all the other extracts were weakly active. Least active extract was that of *T. radiatus* which inhibited only *E. coli followed* by *E. asper* which was weakly active against *P. aeruginosa* and *K. pneumoniae*.

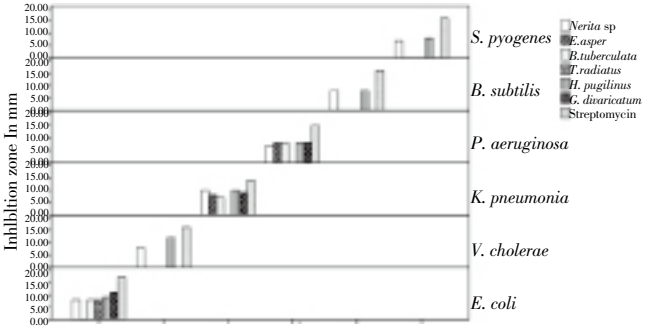

Figure 1. Antibacterial activity of the molluscan extracts (0.5 mg/mL) compared to the positive control of Streptomycin (0.1 mg/mL).

4. Discussion

Table 1
Antibacterial activity of crude methanolic extracts of intertidal molluscs.

Molluscan Species	Bacterial Strains					
	Escherichia coli	*Vibrio cholerae*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	Bacillus subtilis	Streptococcus pyogenes
Nerita sp	+	+	+	+	+	+
Euchelus asper	–	–	+	+	–	–
Bursa tuberculata	+	–	+	+	–	–
Trochus radiates	+	–	–	–	–	–
Hemifusus Pugilinus	+	++	+	+	+	+
Gafrarium divaricatum	++	–	+	+	–	–

(–) No activity, (+) Weak Activity (7– 10 mm halo), (++) Good Activity (10– 15 mm halo)

Table 2
Minimum inhibitory concentration (mg/mL)

Molluscan species	Bacterial Strains					
	Escherichia coli	*Vibrio cholerae*	*Klebsiella pneumonia*	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Streptococcus pyogenes*
Nerita sp	0.3	0.25	0.25	0.25	0.3	0.35
Euchelus asper	–	–	0.25	0.3	–	–
Bursa tuberculata	0.2	–	0.2	0.25	–	–
Trochus radiatus	0.35	–	–	–	–	–
Hemifusus pugilinus	0.2	0.1	0.2	0.2	0.15	0.2
Gafrarium divaricatum	0.1	–	0.2	0.2	–	–
Streptomycin	0.003	0.005	0.002	0.06	0.003	0.003

In this investigation, *Nerita* sp., *H. pugilinus* and *G. divaricatum* have shown promising results which fuels the need to isolate their bioactive compounds and test them against a wider array of pathogens. The considerable activity of *H. pugilinus* against *V. cholerae* and weak activity against *S. pyogenes* is contrary to the observation made by Anand *et al*[8]. In their study, the methanolic extract of *H. pugilinus* inhibited *E. coli*, *B. subtilis* and *K. pneumoniae* significantly while no activity was observed against *V. cholerae* and *S. pyogenes*. This could be due to the variation in the geographic location, food availability and climatic conditions of the organisms tested in both studies. Although earlier *Nerita* sp. had been found to be inactive against bacteria like *E. coli*, *Staphylococcus aureus*, *Streptococcus faecalis*, *K. pneumoniae* and *P. aeruginosa* [13,14], we found it to be weakly active against all the tested bacterial strains. Previously organisms of genus *Nerita* have been shown to exhibit significant activity against biofilm bacteria (15), anti-inflammatory activity[16], as well as presence of an antibacterial pigment "fulvoplumierin" from *Nerita* albicilla by Sanduja *et al*[17]. The bioactivity of methanolic extract of *T. radiatus* against *E. coli* is in confirmation with the earlier reports which, however, observed good bactericidal activity against *V. cholerae* and *B. subtilis* too[18]. Moreover, the *T. radiatus* extract was inactive against *K. pneumoniae* and *P. aeruginosa* in accordance with the previous study by Manilal *et al*[19]. Recent studies have also established antibacterial potential of *Trochus radiatus* against *Staphylococcus aureus* and *E. coli*[20]. Chellaram and Edward[21] also reported anti-inflammatory activity from column-purified acetone fractions of Trochus tentorium. In our study the inactivity of *T. radiatus* extracts can be ascribed to the variation in the secondary metabolites present in the organisms at different points of collection. Earlier Benkendorff[2] has also described variation in antimicrobial activity in molluscan egg masses of different species as well as populations. This intraspecific variability in bioactivity also exists in other marine invertebrates like ascidians collected from different localities[22,23]. This is one of the earliest studies to report the antibacterial potential of *E. asper*, *G. divaricatum*, and *B. tuberculata*. Previously Ramasamy and Murugan[15] had reported antimicrobial activity of *E. asper* and *Gafrarium* sp. against biofilm bacteria.

Commercial antibiotics are significantly effective against all the pathogens while bioactive compounds in marine invertebrates are often present in minute concentrations, sometimes accounting for less than 10%-6% of the wet weight[24]. Hence the MIC of all the bioactive extracts increased to a higher concentration compared to that of the pure antibiotic. The MIC of *T. radiatus* against *E. coli* was found at 0.35 mg/mL of extract whereas in previous study it was found to be much lower (0.07-0.15 mg/mL) against pathogens such as Staphylococcus aureus, *Enterobacter aerogenes*, *Proteus mirabilis* and *Serratia marsescens*[8]. This could be due to the presence of impurities at the time of extraction and purification of current crude extracts. All the extracts displayed moderate biological activity against pathogenic bacteria compared to positive control of Streptomycin. The present study advocates further work on the isolation and identification of the antimicrobial components in these intertidal molluscs for

industrial and pharmaceutical application.

4. Discussion

The current work testifies the bioactive potential of shelled molluscs and provides a baseline data for isolation and characterization of the active constituents. There are very few studies that have involved the marine molluscs used in this investigation. Except *Nerita* sp, *T. radiatus* and *H. pugilinus*, there is little or no data on the other species. All the marine organisms tested here for antibacterial activity are under detailed investigations with the goal of finding seasonal variation in antimicrobial activity. The antimicrobial activity seen in the preliminary experiments reveals the bioactive potential of intertidal molluscs, so the rapid investigation of all available intertidal fauna is prime necessity.

Conflict of interest statement

We declare that we have no conflict of interest.

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