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Antimicrobial Activity from the Gill Extraction of *Perna viridis* (Linnaeus, 1758)

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Abstract: Molluscs are highly delicious seafood and they are also very good source for biomedically imported products. Among the molluscs some have pronounced pharmacological activities or other properties which are useful in the biomedical area. In the present study antimicrobial activity of gill extraction of *Perna viridis* was investigated. In antibacterial activity the maximum zone of inhibition (19 mm) was observed against *Staphylococcus aureus* and minimum activity (11 mm) was observed in *Salmonella paratyphi*. In antifungal activity maximum zone of inhibition was observed in *Aspergillus flavus* (13mm) and minimum zone of inhibition was recorded in *Mucor* sp (11mm). The gill extraction samples showed antimicrobial activity was subjected to SDS-PAGE to estimate the molecular weight of proteins present after electrophoresis only one clear band were detected in the gel which represented proteins of molecular weight 9.7 kDa. In the present study indicated that the gill extraction of *P.viridis* may potential source for antibiotics.

Key words: *Perna viridis* · Gill extraction · Antibacterial · Antifungal · SDS-PAGE

INTRODUCTION

Molluscs are widely distributed throughout the world and have many representatives in the marine and estuarine ecosystem namely slugs, whelks, clams, mussels, oysters, scallops, squids and octopus. Ocean offers a large biodiversity of fauna and flora which is estimated to be over 5, 00,000 species are more than double of the land species [1]. This rich diversity of marine organisms assumes a great opportunity for the discovery of new bioactive substances. Thus the marine environment is an exceptional reservoir for bioactive natural products; many of which exhibit structural features that are not found in terrestrial natural products [2].

From 1960's approximately 300 bioactive marine natural products were field for patent. Approximately 6,500 bioactive compounds have been isolated from the marine organisms [1]. Many classes of bioactive compounds exhibiting antitumour, antileukemia, antibacterial and antiviral activities have been reported world wide [3]. Antimicrobial peptides are important in the first line of the host defense system of many animal species [4]. Their value in innate immunity lies in their ability to function without either high specificity or memory. Moreover their small size make then easy to synthesize without dedicated cells or tissues and they rapidly diffuse to the point of infection. The screening of marine organisms, especially marine bivalves for therapeutic drugs are of greater interest now-a-days. Bivalves are widely used in world research institution for various studies, but only recently they have been recognized as potential sources of antibacterial and antifungal substances. The potential of marine bivalves as a source of biologically active products is largely unexplored. Hence, a broad, based screening of marine bivalves for bioactive compounds is necessary.

MATERIALS AND METHODS

Live specimens of bivalves (*P. viridis*) will be collected from the Vellar estuary of Parangipettai southeast coast of India (Lat 11° 29 'N; 79° 46' E). Mussels was kept on ice until gill tissue isolation and once filaments will dissect than dried with Whatmann paper that was stored at 4° C until use.

Gill Extraction: Gill region were collected by directly the animal with fine scissor. The gill was suspended in methanol for over night then homogenate using clean Mortar and Pestle. Then centrifuge at 5000 rpm for 15 mins the supernatant was collected and stored in refrigerator.

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Microbial Strains Used: Antibacterial activity of gill extraction was determined against 9 bacterial strains viz, *Staphylococcus aureus, Salmonella typhi, S. paratyphi, Klebsiella oxytoca, K. pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis* and *Lactobacillus vulgaris* and 6 fungal strains viz., *Aspergillus niger, A. flavus, Candida albicans, Rhizopus, Cryptococcus neoformans* and *Mucor* sp. These clinical strains were obtained from the Department of Medical Microbiology (Raja Muthiah Medical College hospital) Annamalai University, Annamalai Nagar.

Anti Microbial Assay: The spectrum of antibacterial and antifungal activity was investigated by using the standard techniques [5]. Antibacterial and antifungal activity was expressed in terms of diameter of Zone of inhibition was measured in mm using Vernier caliper or a scale and recorded.

SDS-PAGE: SDS-PAGE was performed in 12% separating gels, according to the standard method [6]. The reference proteins for molecular weight estimation were different molecular weight marker proteins were used in β-Gatactosidase (116kDa), Fructose-6-phosphate kinase (85.2 kDa), Glutamate dehydrogenase (55.6 kDa), Trypsin inhibitor (20.1 kDa) and Lysozyme (14.3 kDa). The electrophoresis was carried out at constant 100 volts for 3 hours. Following electrophoresis, the protein, the protein bands were visualized by staining with Coomassie Brilliant-250.

RESULTS

Antimicrobial Assay: The zone of inhibition in different bacterial strains against *P. viridis* gill extraction is shown in (Fig. 1). Among the various strains maximum zone of inhibition (19 mm) was recorded in *S. aureus* strain

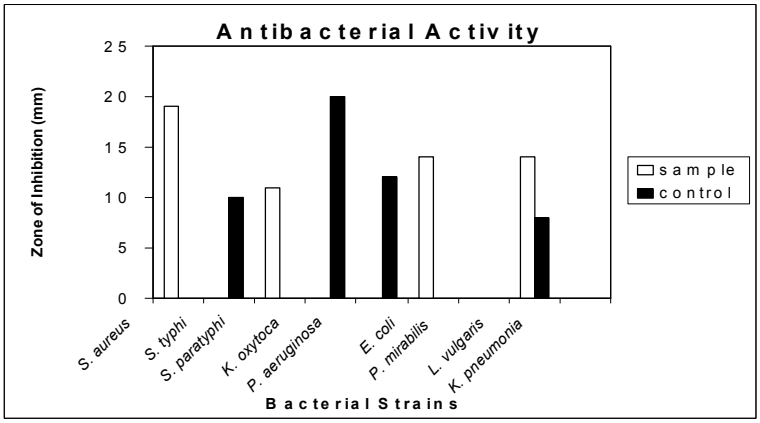


Fig. 1: Antibacterial activity of positive control of Erythromycin and *P.viridis*

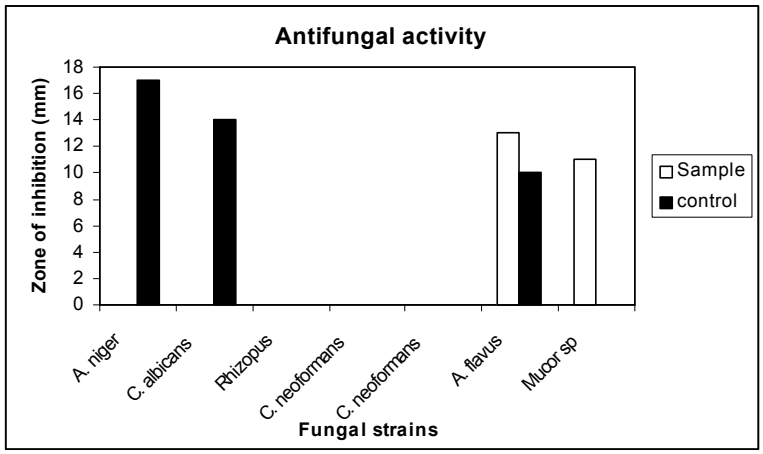
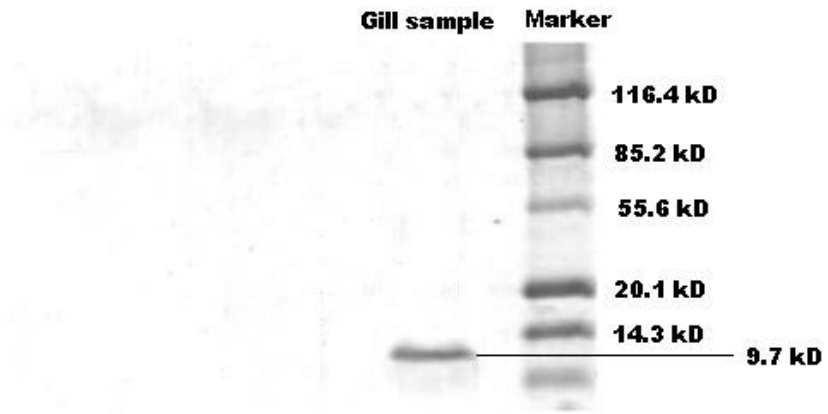


Fig. 2: Antifungal activity of positive control of fluconazole and *P.viridis*



Lane M: Molecular weight marker proteins
β-Galactosidase (116.4 kD)
Fruclose -6-phosphate--kinase (85.2kD)
Glutamate dehydrogenase (55.6kD)
Trypsin inhabitor (20.1kD)
Lysozyme (14.3kD)

Fig. 3: SDS PAGE result of active fraction of the gill extraction of *P. viridis*

and minimum zone of inhibition (11 mm) was observed in *S. paratyphi* strain. The positive control (Erythromycin) was showed activity against all the bacterial strains tested. The maximum activity against *K. oxytoca* (20mm) and the minimum activity were observed against *L. vulgaris* (08mm).

The antifungal activity of the gill extraction shows maximum activity in *Aspergillus flavus* (13mm) and minimum activity was recorded in *Mucor* sp. (11mm) is shown in (Fig.2). The positive antifungal agent (Fluconazole) showed activity against all the fungal strains tested. The maximum activity was observed against *A. niger* (17mm) and the minimum activity was recorded against *Aspergillus flavus* (10mm).

SDS-PAGE: The gill extraction samples showed antimicrobial activity was subjected to SDS-PAGE to estimate the molecular weight of proteins present in it. Different standard were used to determine the molecular weight of gill extract proteins. The stained gel revealed that the gill extract contained a simple population of proteins. There is different molecular weight marker proteins were used only one clear band was detected in the gel that represented peptide of 9.7 kDa shown in (Fig. 3).

DISCUSSION

In the present investigation a pronounced antimicrobial activity has been observed against some bacterial and fungal strains. The methanol fractions of *P. viridis* gill extraction shows activity against both bacterial and fungal strains. In antibacterial activity the maximum zone of inhibition was recorded in *S. aureus* strain and minimum zone of inhibition was observed in *S. paratyphi* strain. The maximum antifungal activity was observed against *Aspergillus flavus* and minimum activity was recorded in *Mucor* sp. Similar result was reported in four bivalves against few pathogens and found that extracts showed significant activity against *Bacillus subtillus* [7]. Like wise highest activity was observed against *S. typhi* in some gastropods [8]. These investigations lend support to the present findings of the antimicrobial activity of gill extraction of *P. viridis*.

The antibacterial activities of ethanol extracts of gastropods *Babylonia spirata* and *Turbo brunneus* was observed maximum activity against *E. coli, K. pneumoniae, P. vulgaris* and *S. typhi* [8]. This study corroborates the results of the present investigation. Very similar to this maximum antibacterial activity against *S. aureus* and *E. coli.* on *Trochus radiatus* was reported [9].

The first attempt to locate antimicrobial activity in marine organisms was initiated around 1950's [10]. Since this time, a large number of marine organisms from a wide range of phyla have been screened for antimicrobial activity [11]. Many of these organisms have been 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 obtained from microorganisms [12].

The presence of antimicrobial activity in mollusca has been reported from the mucus of the giant snail *Achantina fulica* [13,14], from the egg mass and purple fluid of the sea hare *Aplysia kurodai* [15,16] and from the body wall of the sea hare *Dolabella aurigularia* [17]. Work on marine mollusks has focused on the mussels *Mytillus edulis* antibacterial and antifungal peptides. Two antimicrobial peptides, defensins A and B were purified that were close in sequence and show a high degree of similarity with arthropod defensins, a large family of cysteine-rich cationic peptides. The positions of the cysteines in arthropod defensins are highly conserved and this array is identical to that of defensins A and B from *Mytillus edulis* [18].

Mytillins A and B, cationic cysteine-rich antimicrobial peptides, were isolated and fully characterized from *Mytillus edulis* and showed no homology with known peptides in the peptide sequence database [18]. The Mytilin isoforms C, D and G1 were isolated from *Mytillus galloprovincialis* and exhibited complementary antimicrobial properties [19]. The mytilins are notably rich in cysteine residues with respect to their small size, indicating that their three dimensional structure is highly compact [18]. But the connectivity of their disulfide bonds has yet to be determined. In addition, a novel antifungal peptide that delays the growth of *Neurospora crassa* and *Fusarium culmonum*, Mytimycin shows no homology with reported peptides sequence in protein databases. In the present investigation gill extraction that showed antimicrobial activity was subjected to SDS-PAGE to estimate the number and molecular weight of proteins present. After electrophoresis clear band were detected in the gel which represented proteins of molecular weight 9.7 kDa. In conclusion in the present study indicates that the gill extraction of *P. viridis* would be a good source of antimicrobial agents and would replace the existing inadequate and cost effective antibiotics.

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