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Research Article

# ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF THE TISSUE EXTRACT OF *PERNA VIRIDIS* LINNAEUS, 1758 (MOLLUSCA: BIVALVIA) FROM VERSOVA COAST, MUMBAI

**MADHU V. N[1&3], P. SIVAPERUMAL*[2], K. KAMALA[2], AJIT A. AMBEKAR[2] AND B.G. KULKARNI[3]**

**[1]Patkar College of Arts and Science, Mumbai 62, [2]Central Institute of Fisheries Education, ICAR - Deemed University, Mumbai 61, [3]Institute of Science, Mumbai 32. Email: marinesiva86@gmail.com**

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**ABSTRACT**

Objective: The study of marine organism for their bioactive potential, being a significant part of marine ecosystem has picked up the regularity in recent years with the growth recognition of their importance in human life as well as animals.

Methods: In this present study methanol tissue extract of edible green mussel (*P. viridis*) was assayed for the antibacterial activity against six bacterial pathogens and antioxidant activities also determined.

Results: The antibacterial activity of *P. viridis* tissue extract, showed maximum zone of inhibition (15mm) against *Vibrio cholerae* and minimum activity (5 mm) was observed in *Klebsiella pneumoniae*. Molecular size of green mussel protein was determined using Sodiumdodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). FTIR analysis reveals the presence of bioactive compounds signals at different ranges. The antioxidant activity of crude protein tissue extract from the *P. viridis* were measured in different system of assay such as DPPH assay (76.9%), total reducing power (27.8 µg), total antioxidant activity (174 µg), hydrogen peroxide scavenging (88.12 %) and nitrous oxide scavenging activity (62.5%) at 100µg/ml.

Conclusion: crude tissue extract from *P. viridis* could be effectively used as alternative source of antimicrobial and antioxidant with subsequent health benefits.

**Keywords:** Green mussel, Antibacterial, FT-IR, SDS-PAGE and Antioxidant.

## INTRODUCTION

A wide range of bioactive substances are being isolated and characterized from the food that is derived from the marine environment, several with enormous assure for the treatment of various diseases. Marine organisms are a wealthy resource of structurally novel and biologically active metabolites. So far, frequent chemically exclusive compounds of marine origin with different biologically activity have been isolated and a number of them are under investigation and/ or are being developed as new pharmaceuticals [1, 2, 3 & 4]. For the past two decades pharmaceutical industry has been relatively successful in overcoming problems due to single resistant determinants. In recent years, natural products from marine samples have a broad variety of biological activities and abundant therapeutic applications contain antiviral, antibacterial, antitumor activity and very different kinds of substances have been obtained.

In marine invertebrates so far 7, 000 marine natural products have been reported, from sponges (33%), coelenterates (18%) sea whips, sea fans and soft corals (24%) from representatives of other invertebrate phyla molluscs (nudibranchs, sea hares, etc), echinoderms (starfish, sea cucumbers, etc) and bryozoans (moss animals) [5]. The marine animals cyclic and linear peptides discovered have increased our acquaintance about new effective cytotoxic, antimicrobial, ion channels particular blockers, and many other properties with novel chemical structures associated to original mechanisms of pharmacological activity [6]. There is an increasing curiosity in antioxidants particularly in those of free radicals in different diseases.

These pathological and clinical backgrounds have encouraged to investigate novel and persuasive antioxidant peptides from bivalve which are ultimately of therapeutic use. A lot of studies on bioactive compounds from molluscs exhibit antitumor, antileukemic and antiviral activities have been reported worldwide [7, 8 & 9]. The marine bivalves are very good source for human consumption as well as bioactive compounds. Therefore, the aim of the present study was to assess the antibacterial and antioxidant activities of the tissue extract of edible green mussel (*P. viridis*).

## MATERIALS AND METHODS

### Collection of animal and preparation of crude extract

The animals were collected from Versova rocky shore area at low tide level and collected animal brought to the laboratory. The shells were removed and the tissue samples were washed with distilled water. The presence of bioactive compounds was extracted with methanol solvent (10g of tissue sample was ground well with 10 ml of methanol using mortar and pestle). The extract was centrifuged at 10000 rpm for 30 min and the supernatants were collected and concentrated by rotary evaporator with reduced pressure to give predominantly an aqueous suspension and freeze dried to give yellow gummy mass and stored at -20 º C until use.

### Protein estimation and Molecular weight determination

The protein content of crude methanol extract was estimated by Lowry's method using Bovine serum albumin as a standard [10]. The molecular weight distribution in the protein was determined by SDS-PAGE, according to Lammeli *et al.*[11]. SDS-PAGE was performed in 10% separating gels and 5% stacking gel.

### Characterization of protein by FT-IR spectroscopy

The lyophilized crude extract of *P. viridis* was subjected to FT-IR analysis. The IR spectrum of the protein was recorded with a Perkin-Elmer model 297IR spectrophotometer. One part of the crude extract was mixed with 99 part of dried potassium bromide and it was scanned between 600 – 4000 wave number ($cm^{-1}$) at a speed of 1 micron/ min and with a programmed slit opening and air as reference.

### Antibacterial assay

*In-vitro* antibacterial assay was carried out by disc diffusion method [12]. The following bacterial strains were used for the antibacterial activity as follows: *Escherichia coli (E.coli), Salmonella typhi, Klebsiella pneumoniae, Vibrio parahaemolyticus, Vibrio cholerae,* and *Staphylococcus aureus.* The bacterial strains were maintained in nutrient agar. Inoculums were prepared using fresh culture of bacterial strains.

A loop of bacterial culture was inoculated into a nutrient broth. 100µg/ml concentrations of crude extract were impregnated in filter paper (Whatmann No.1) disc with 5mm diameter. The impregnated discs along with positive control (streptomycin) and negative control (methanol solvent) were kept at the nutrient agar plates. All the plates were incubated at 37˚C for bacteria. The zone of inhibitions was measured after 24 h of incubation.

**Antioxidant activity**

The antioxidant activity of the protein from the edible green mussel of *P. viridis* was evaluated in terms of DPPH assay, total reducing power, total antioxidant activity, hydrogen peroxide scavenging and nitrous oxide scavenging activity followed by [12].

**RESULTS**

**Protein estimation and Molecular weight determination**

The amount of protein present in the crude methanol extract was found to be 300µg/mg of the sample. In the case of molecular weight resolve, the electrophoretic profile of the samples showed the presence of small to high molecular weight protein; upon these some of them are distinct. Following SDS–PAGE, under non-reducing conditions 5 bands were seen along the gel in crude extract. The sample shows intensive bands at 54 kDa, 48 kDa and 29 kDa some of the indistinct band also observed at 63 kDa and 36 kDa (Fig.1).

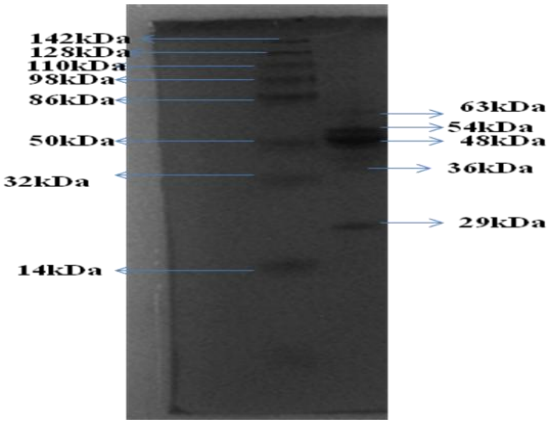


**Fig. 1: Electrophoretic profile of the *Perna viridis* tissue extract was analysed using 15% SDS-PAGE. Gel was stained with coomassie Blue. Lane 1 corresponds to the position of molecular mass markers and Lane 2 was extract of *P. viridis***

**Characterization of protein by FT-IR spectroscopy**

Infrared spectroscopy of the crude protein extract of *P.viridis* was recorded using Bio-Rad FTES-40 equipment. The crude protein spectra showed 14 peaks. The FT-IR spectrum of protein revealed that the peak 3411 indicates the presence of NH stretching coupled with HI. Similarly the wave number, 1500 indicate the bending of NH coupled with CN stretch

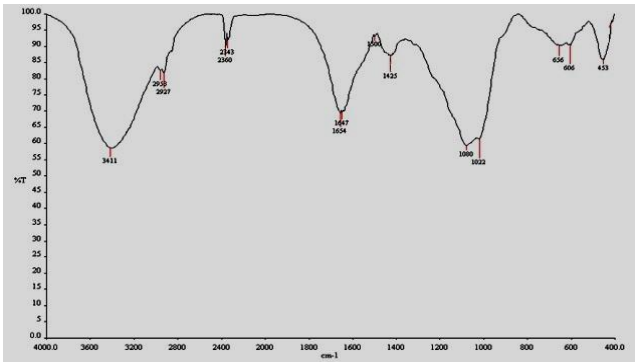


**Fig. 2: FT-IR- spectra represented to crude protein from *P. viridis* tissue extract**

The wave numbers 2958, 2927, 2360 and 2343 distinctive of asymmetrical stretching of $CH_2$ and 1654, 1647, 1022 and 1000 and 656-453 positions of the spectrums are the characteristic C=O stretching, COH, CH, C-O and Skeletal stretch respectively. These asymmetrical stretching and skeletal stretch indicated that the being there of the amide groups (Table 1, 2 & Figure 2). Among the peaks, ten were found to be major and were indicating amide groups (Amide I - 1654$cm^{-1}$ to 1647$cm^{-1}$, Amide II 1500$cm^{-1}$, Amide III 1425$cm^{-1}$, Amide IV 656$cm^{-1}$ to 453$cm^{-1}$, Table 2).

**Table 1: Crude tissue extract from *P.viridis* sample in FT-IR analysis**

| **Crude extract from *P.viridis*** | |
|---|---|
| **Band position ($cm^{-1}$)** | **Assignment** |
| 3411 | NH stretch, coupled with HI |
| 2958-2927 | CH2 assymmetrical stretch |
| 2360-2343 | CH2 assymmetrical stretch |
| 1654-1647 | C=O stretch |
| 1500 | NH bend coupled with CN stretch |
| 1425 | COH |
| 1022 – 1000 | C-O |
| 656-453 | Skeletal stretch |

**Table 2: Represented functional groups of tissue extract from *P. viridis* sample in FT-IR analysis**

| **Band position ($cm^{-1}$)** | **Functional Groups** |
|---|---|
| 3411$cm^{-1}$ | Amide A |
| 2958 - 2343 $cm^{-1}$ | Amide B |
| 1654 $cm^{-1}$ | Amide I(α- helical) |
| 1647 $cm^{-1}$ | Amide I(Random coil) |
| 1500 $cm^{-1}$ | Amide II |
| 1425 $cm^{-1}$ | Amide III |
| 656-453 | Amide IV |

**Antibacterial assay**

The antibacterial activity of tissue extract of *P.viridis* was shown in Table.3. The zone inhibition varied from 5 to 15 mm. Maximum diameters was noted against *Vibrio cholerae* (15mm) and minimum diameters was recorded against *V. parahaemolyticus* (12mm) followed by *Escherichia coli* (10mm), and *Klebsiella pneumoniae* (5mm). The ranges of MIC varied between 20µg/mg - 50µg/mg.

**Table 3: Antibacterial activity of tissue extract of *P.viridis***

| **Test organisms** | **Inhibition zone (mm)** | **MIC (µg/mg)** |
|---|---|---|
| *Escherichia coli* | 10 | 35 |
| *Salmonella typhi* | - | - |
| *Klebsiella pneumoniae* | 5 | 50 |
| *V.parahaemolyticus* | 12 | 30 |
| *Vibrio cholerae* | 15 | 20 |
| *Staphylococcus aureus* | - | - |

**Antioxidant activity**

The antioxidant activity of crude protein issue extracts from the *P. viridis* were measured in different system of assay such as DPPH assay, total reducing power, total antioxidant activity, hydrogen peroxide scavenging and nitrous oxide scavenging activity. The crude protein was found to have different levels of antioxidant activity in different concentrations (20, 40, 60, 80 & 100 µg/ml) tested. The free radical scavenging activity of protein from green mussel *P. viridis* extract was assessed by the DPPH assay. These assay shows that a significant decrease in the concentration of DPPH radical due to scavenging ability of the protein. The result shows that crude tissue extract from green mussel had the significant DPPH scavenging activity (76.9%) at 100µg/ml. The reducing capacity of *P.viridis* crude protein compared to standard Ascorbic acid. Green mussel tissue extract (100µg/ml) showed reducing ability in 27.8 µg.

These assay compared with standard Ascorbic acid equivalent. The Total antioxidant activity of protein of *P. viridis* was assessed by the formation of green phosphate complex at acidic pH lustrate that the antioxidant capacity of the protein (20-100 µg/mg) with standard ascorbic acid. The result shows that crude extract had the potential activity. This indicates that the crude protein of mussel tissue sample has a good source of natural antioxidants. The scavenging effect of hydroxyl radical was investigated using the fenton reaction and the results were shown as an inhibition rate. The crude tissue extract of *P. viridis* exhibited the highest inhibition of about 88.12 % at the concentration of 100µg/ml. Suppression of Nitric oxide free radical release may be attributed to a direct nitric oxide free radical scavenging effect to decrease the amount of nitrite generated from the decomposition of sodium nitroprusside. The result shows that green mussel protein had scavenging activity 62.5% at the concentration of 100µg/ml compared with the standard Ascorbic acid (Figure 3).

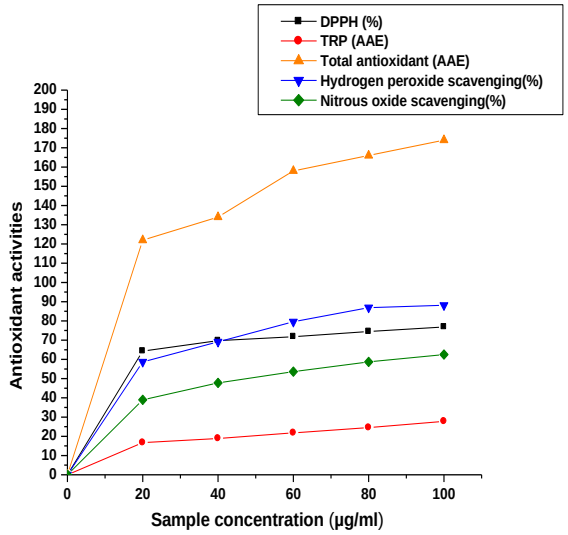


**Fig. 3: Antioxidant activities of crude extract from green mussel *P. viridis***

## DISCUSSION

There is a rising interest in marine natural products and marine secondary metabolites. This field of study receives the attention of investigators from various disciplines such as marine biology, biochemistry, pharmacology and biotechnology.

The studies carried out with marine natural products during the last decades have uncovered many substances with biomedical potential, which has raised the significance of many research groups toward this ecosystem as a source of new drugs [13]. Many antimicrobial screening studies have shown that the Gram negative bacteria are more sensitive than Gram positive bacteria [14].

Overall, the gastropod extract caused growth inhibition in gram positive and gram negative bacteria. Thus, indicating to facilitate this extract does not selectively inhibit one group of microorganism. In the present study, a prominent antibacterial activity has been observed against some bacterial strains.

The highest antibacterial activity was observed in *Vibrio cholerae* (15mm) and minimum diameters was recorded against *V. parahaemolyticus* (12mm) followed by *Escherichia coli* (10mm), and *Klebsiella pneumoniae* (5mm). Previously, the antimicrobial activity was reported in four bivalves against few pathogens and found that extracts showed significant activity against *Bacillus subtilis* [15]. Further the maximum antibacterial activity was reported in *Trochus radiates* against *S.aureus* and *E.coli* [16]. The antimicrobial activity of *Babylona spirata* was also shown mild activities [17]. The antimicrobial activity from the gill extraction of *P. viridis,* tissue extract of *Meretrix meretrix*, *M. casta* and edible oyster *Crassostrea madrasensis* were reported [18]. Compared to these studies, the methanolic extract of *P. viridis* shows higher degree of inhibition indicated that the substance involved in producing the antibacterial effect could be a high polar compound. In present study, the molecular weight determination of crude tissue extract from *P. viridis* range from 63 kDa to 29 kDa. These revealed proteins may be responsible for various biological activities in the tissue extracts. More or less similar molecular weight protein was also isolated from [17] 14 KDa and 29 KDa in marine bivalves *M. casta* and *P. viridis*.[18] reported that the crude proteins showed 5 to 6 bands ranging from 45 to 261 KDa on *Meretrix meretrix* and *Meretrix casta*. In the present study indicates that the tissue extraction of *P. viridis* would be a good resource of antibacterial compounds and would substitute the existing inadequate and cost effective antibiotics.

IR spectrum of the crude antibacterial methanol extract of *P. viridis* was compared with standard antibiotic. The IR spectrum of the standard depicted 14 peaks which is said to be responsible for the chemical groups. The band at 1654 and 1647,cm-1, which occurs at similar wavelength in polyamides and proteins, is commonly assigned to stretching of the C=O group hydrogen bonded to N-H of the neighboring infra sheet chain [19]. The acetyl amino group was represented by a band at 1022 cm-1, 1000 cm-1 asymmetric in phase ring stretching mode and 656 cm-1 OH-Out-of plane bending. [20]. The present observation of crude protein peaks are similar to bands in the 820-850 cm-1 spectral region were attributed C-O-S stretching based on the results of [21], as observed by [22] the sample showed the absorption band for the carboxylic group at same peak at 1654 cm-1 and acetyl amino group at 1400 cm-1 which were also reported by [23] that 1615 cm-1 (carboxylic group) and 1375 cm-1 (acetyl amino group) in the sulfated mucopolysacharides isolated from the skin of *chimaera* sp. FTIR analysis reveals the occurrence of antimicrobial compound signals at different ranges. The present investigation the bivalve muscle is value medicinal due to high quality of antimicrobial compounds. Crude products isolated from marine organisms have served as a basis of drugs and preliminary materials for synthesis of useful drugs. In addition, because of the differences in the environmental conditions, new or remarkable biochemical entity having biological activity can be evolved by marine organisms. So it is understood that the studies of new and unique compounds derived from marine organisms will continue to increase our fundamental knowledge with respect to pharmacology and medicine. The results in this study show that green muscle *P. viridis* tissue of crude protein sample is significance drug due to high quantity bioactive compound, well-balanced antibacterial activity. In conclusion in the present study indicates that the crude tissue extracts from *P. viridis* could be effectively used as alternative source of antimicrobial and antioxidant with subsequent health benefits.

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