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Marine Gastropod *Planaxis sulcatus* (Born, 1778) of Uvari Coastal Waters: A Comprehensive Study on Its Antibacterial Activity, Biochemical Composition and FTIR Spectroscopy

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Diverse nutritional profiles and potent antimicrobial properties distinguish marine gastropods from other members of the phylum Mollusca. This study targets the investigation of the antibacterial activity, biochemical and FTIR analysis of ethanol extracts of marine gastropod mollusc *Planaxis sulcatus*. The well diffusion method was used to carry out the antibacterial assay. The extract showed the maximum inhibition (21±2 mm inhibitory zone) against the human bacterial pathogen *S.*

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aureus, whereas *K. pneumonia* showed the lowest susceptibility (12 mm). The extracts had MIC values between 0.21 and 1.04 mg/ml. The biochemical assay's findings revealed that the majority of the snail's flesh (51%) is made up of protein, with only 5% of the mass coming from carbohydrates and very trace amounts of fat (0.2%). The FTIR analysis represents the presence of alkyl halides, nitro compounds, aromatic compounds, alkynes, nitriles, carboxylic acid, aldehyde, alkanes and alcohol in the gastropod extracts. The aforementioned observational result demonstrates the abundance of antibacterial substances in the gastropod extracts. Further research is necessary to fully understand the structural composition of the antibacterial substances found in this marine snail.

Keywords: Marine molluscs; biochemical composition; antimicrobial activity and FT-IR.

1. INTRODUCTION

The development of new, more targeted and effective medications from naturally occurring bioactive chemicals is one of the main goals of modern research (Kirilova et al. 2024). Marine gastropods have emerged as one of the most promising groups of marine organisms for drug discovery, because they live in complex habitats and are exposed to harsh environments. This is because their habitats produce a wide range of highly concentrated and specialized bioactive chemicals that are specific to them (Hamed et al. 2015). Thousands of unique compounds have been discovered to date, illustrating the extraordinarily wide diversity of chemical characteristics produced from marine gastropods (Datta et al. 2015). These substances are helpful instruments in the medical field since they can be used as molecules with antibacterial, antifungal, antiviral, and other qualities (Khan and Liu 2019). The extraction of bioactive compounds from molluscan species has received little attention even though they have high biodiversity (Ngandjui et al. 2024). Therefore, the purpose of this work is to report on the antimicrobial, biochemical, and FTIR analysis of the crude ethanol extracts of *Planaxis sulcatus* (*P. sulcatus*), a gastropod mollusc that was obtained from the sea of Uvari in Tirunelveli, Tamilnadu. *P. sulcatus* is a rocky seashore gastropod mollusc which is a member of the *Planaxidae* family.

2. MATERIALS AND METHODS

2.1 Collection and Identification

The samples of *P. sulcatus* (Fig. 1b.) were collected by handpicking at Uvari coastal waters (Latitude: 8.16'42 N; Longitude: 77°.53'38 E) of Tirunelveli District, Tamilnadu (Fig. 1a.). After being thoroughly cleansed with seawater to get rid of any debris, the samples were brought to the lab. The samples were preserved in 90% ethanol and delivered to Zoological Survey of India (ZSI) for identification. The specimens were identified as *Planaxis sulcatus* (Born, 1778) using standard keys of ZSI (Subba Rao 2003) and the voucher specimens were deposited in ZSI, Pune.

2.2 Solvent Extraction

After brought to laboratory the snails were carefully washed with tap water and the soft tissues were taken out of their shells and collected in sterilized containers. Using a mixer grinder, the tissue was blended and then extracted with ethanol at a 1:4 (g/ml) ratio. After being cold steeped for 72 hours at 4°C, the extracts were filtered through Whatman No. 1 filter paper. After allowing the filtrate to evaporate to a third of its original volume, the crude extracts were refrigerated until they could be further examined

2.3 Biochemical Evaluation

The protein content was analysed by Biuret method (Gornall et al. 1949) by using bovine serum albumin as standard. Carbohydrate estimation was done by Anthrone reagent method (Seifter et al. 1950). Lipid estimation was done by chloroform-methanol method (Folch et al. 1957).

2.4 Antibacterial Assay

2.4.1 Inoculum Preparation

Four species of human pathogens; two Gram-negative bacteria such as *E.coli, Klebsiella pneumonia* and Gram-positive bacteria *Enterococcus faecalis, Staphylococcus aureus* and were obtained from the Scudder Lab, Nagercoil. The bacterial strains were individually inoculated in sterilized nutrient broth and were incubated at 37°C for 24 hours and used in the antibacterial assay.

Fig. 1a. Map showing study area; b. Image of study animal *P. sulcatus*

2.4.2 Agar well diffusion method

The antibacterial properties of the sample extracts were analysed by the well diffusion technique (Ramasamy et al. 2011). The 24 hours old test bacterial cultures in nutrient broth were aseptically swabbed onto sterile nutrient agar plates. Using a sterile cork borer, the 6 mm diameter wells were punched onto the nutrient agar plates. An extract volume of 10–50 µL was added to each well. The positive control was 25 µL (10 mg/ml) of streptomycin, while the negative control was the solvent. For 24 hours, plates were incubated at 37°C. The zone of inhibition was measured in millimeters to assess the antibacterial properties. The assays were performed in triplicates, and the results were reported as Mean ±Standard Deviation.

2.4.3 MIC

The minimum inhibitory concentration (MIC) was assayed using the micro broth dilution test (Sarker et al. 2017). In a microtiter plate filled with nutrient broth, twofold serial dilutions were made directly to create different concentrations. In each well, the bacterial inoculum was added until the final concentration reached 5×10^5 CFU/mL. Streptomycin was used as the positive control. A sterile lid was placed over the plate, and it was incubated at 37°C for 24 hours. Followed by Resazurin solution was added to each well and incubated for 30 minutes at 37°C. The colour of the wells with bacterial growth changed to pink, while the wells without growth stayed blue. The minimum inhibitory concentration (MIC) was identified as the lowest concentration of extract at which bacterial growth is totally inhibited.

2.5 FTIR Analysis of Compounds

About 10µg of dried ethanol extract of *P. sulcatus* was mixed with 100 µg of dried potassium bromide (kbr) and compressed to prepare as a salt disc. The disc was then allowed to read using spectrophotometer (Shimadzu, IR Affinity) with absorption frequencies ranged from 400-4000cm^{-1}. The peak values of extract were recorded and functional groups of molecules were determined.

3. RESULTS AND DISCUSSION

Marine gastropods are rich in nutrients. The biochemical composition of the marine snail *P. sulcatus* is depicted in Fig. 2. The biochemical assay revealed that the gastropod's tissue has a very small quantity of lipids (0.3%) and is high in protein (51%) and low in carbohydrates (5%). Some previous studies revealed that the marine gastropods *Babylonia zeylanica*, *Murex virgineus, B. spirata*, and *Trochus radiatus* had elevated levels of protein concentrations ranging from 43% to 28% (Margret et al. 2013). Additionally, *Turbo bruneus* had a carbohydrate content of 5 to 8%, while *B. spinosa*, a mesogastropod, had a content of 3 to 8% (Babu et al. 2010). Furthermore, extremely low levels of lipid were found in some gastropods, including *Haliotis discus hannai* (0.3%), *B. areolata* (0.8–1.0%), *B. spirata* (0.3%), and *Chicoreus ramosus* (2%) (Abdullah et al. 2016, Aziz 2016, Govindarajalu et al. 2016, Jang et al. 2010) but some turban snails are rich in fat (Ab et al. 2017). It was shown that the types of organisms, body parts, seasonal fluctuations, collecting sites, spatial changes, variations in time, and reproductive cycles all had an impact on the

nutritional profiles of the gastropods (Smoothey 2013).

Figs. 3-4 illustrate the antibacterial effects of marine gastropod *P. sulcatus* tissue extracts against four human pathogens. Streptomycin, the positive control, inhibited all pathogens with the highest activity observed in *E. faecalis* (42 mm), and the lowest activity in *E. coli* (30 mm), *K. pneumoniae* (30 mm), and *S. aureus* (25 mm). No inhibition was observed in the negative control. When tested against *S. aureus*, the extract showed a maximal inhibitory zone of 21±2 mm. It was succeeded by 15.67±1.15 mm, 15±1 mm, and 12 mm against *E. coli, E. faecalis* and *K. pneumonia* respectively. Numerous earlier studies on different gastropods produced similar findings. For example, the snail *T. radiates,* had the greatest antibacterial action against *S. aureus* and *E. coli* (Elezabeth et al. 2003). Similarly it was reported that the growth of *S. aureus, K. pneumoniae, E. faecalis,* and *P. aeruginosa* was effectively prevented by the ethanol extracts of the gastropods *T. brunneus, Mauritia arabica, Pururа bufo,* and *Pleuroploca trapezium* (Anita et al. 2020). Likewise, *B. spirata* and *Chicoreus ramosus* crude ethanol extracts showed high effectiveness against *P. aeruginosa* (Periyasamy et al. 2012) and *P. vulgaris* (Giftson and Patterson 2016).

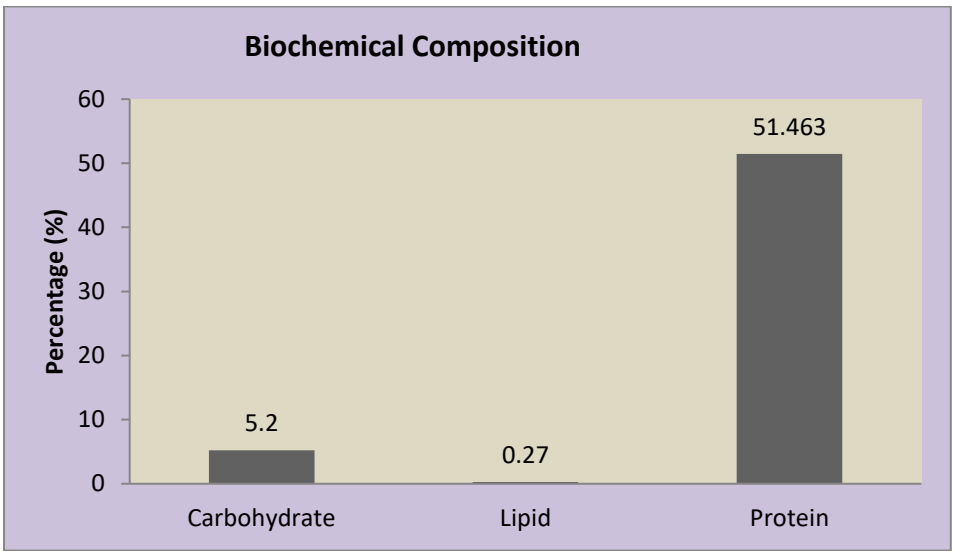

Fig. 2. Biochemical composition of *P. sulcatus* expressed in percentage

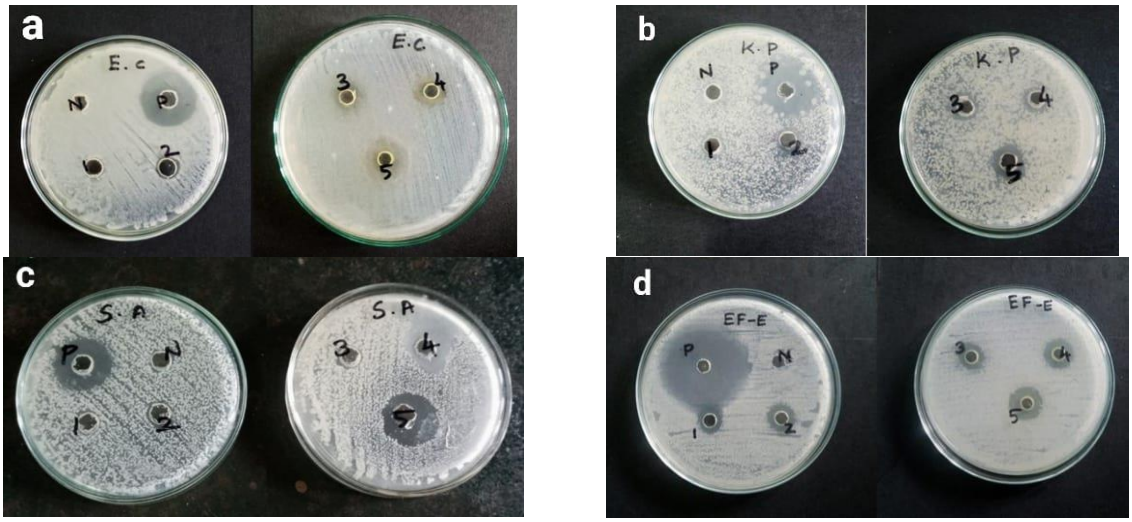

Fig. 3. Antibacterial activity of *P. sulcatus* against some human pathogens (a- *E. coli*; b- *K. pneumonia*; c- *S. aureus*; d- *E. faecalis*) (P- positive control; N- negative control; 1-10µl; 2-20 µl; 3-30 µl; 4- µl; 5- 50µl of extract)

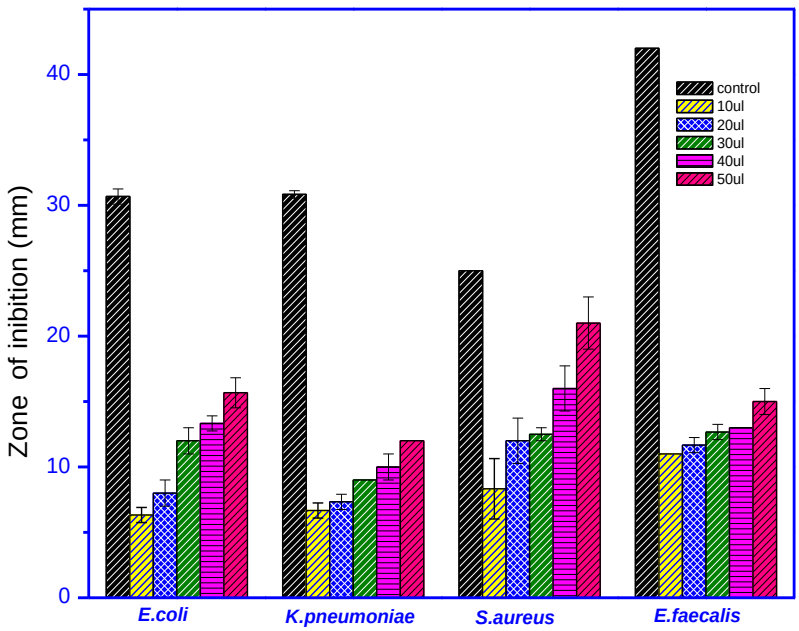

Fig. 4. Antibacterial activity of crude ethanol extracts of *P. sulcatus* against some human pathogens

Table 1. MIC values of ethanol extracts of *P. sulcatus* against human pathogens (mg/ml)

Bacteria	MIC (mg/ml)
E. coli	0.63
K. pneumoniae	1.04
S. aureus	0.21
E. faecalis	0.73

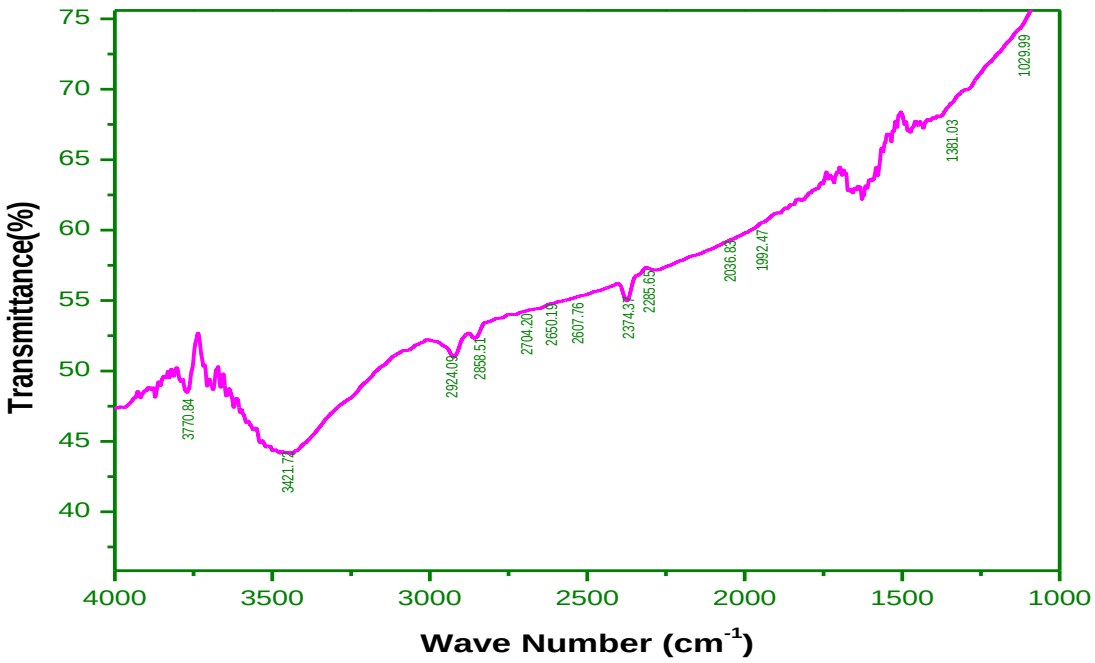

Fig. 5. FT-IR graph of crude ethanol extract of the *P. sulcatus*

Table 2. FT-IR analysis of crude ethanol extract of soft tissue of *P. sulcatus*

Wavenumber	Functional group	Bioactive functional group
1029.99	C-F Stretch	Alkyl halides
1381.03	N–O asymmetric stretch	Nitro compounds
1992.47	C -H Bending	Aromatic compounds
2036.83	C-C stretching	Alkynes
2285.65	C≡N stretch	Nitriles
2374.37	NH Stretching	Amino acid
2607.76	O-H Stretching	carboxylic acid
2650.19	CH stretching vibration	Alkynes
2704.20	C-H stretching	Aldehyde
2858.51	C-H	Alkanes
2924.09	O–H stretch	Carboxylic acids
3421.72	–OH stretching	Alcohol
3770.84	O-H	Alcohol

Table 1 displays the minimum inhibitory concentration (MIC) of *P. sulcatus* ethanol extract. The minimum inhibitory concentration (MIC) of *P. sulcatus's* ethanol extract against human pathogens, including *S. aureus, E. coli*, *E. faecalis* and *K. pneumonia* was reported to be 0.21, 0.63, 0.73 and 1.04 mg/ml respectively.

The FTIR spectra formed characteristic bands in the frequency range between 1029 cm^{-1} and 3770 cm^{-1}. FTIR spectrum showed that 12 major peaks and were showing the presence of alkyl halides, alkynes, alkanes, carboxylic acids, alcohol, nitriles and nitro compounds as functional groups (Table 2; Fig. 5). The previous research showed that the *B. spirata* from Thazhagunda southeast coast of India also recorded the number of peaks lying between 465.75cm-1 to 3388.75cm-1 (Periyasamy et al. 2012). Hence this study confirms that the marine gastropod *P.sulcatus* is highly valuable as therapeutic animals due to the abundance of bioactive chemicals and so it can be used for the development of antibiotics in future research.

4. CONCLUSION

According to the current investigation, *P. sulcatus*, a marine snail, exhibited antibacterial activity against harmful microbes. The investigated gastropod species may be regarded as a biological source of bioactive chemicals and can be utilized for the manufacture of novel antibiotics for bacterial infections since antibacterial compounds from natural resources would be the alternative to overcome the resistance concerns. However, additional investigation is required to pinpoint, describe, and isolate the chemical constituents that give this animal its antibacterial properties.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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