CHITOSAN FROM SHRIMP SHELLS AND TUNIC OF ASCIDIAN: EXTRACTION AND ANTIBACTERIAL POTENTIAL AGAINST PATHOGENS

J. Jaya Abirami, M. Paripooranaselvi

PG and Research Department of Zoology, St. Mary's College (Autonomous), Thoothukudi, Affiliated to Manonmaniam Sundaranar University, Thirunelveli, Tamil Nadu, India

ABSTRACT

Chitosan is a naturally derived biopolymer obtained from chitin, the second most abundant polysaccharide in nature after cellulose and is present in the crustacean exoskeleton (shrimp, crabs, and lobsters), insect cuticles, fungal cell walls, and certain marine organisms such as ascidians (tunicates). The aim of this work was to separate chitosan from shrimp shell and *Phallusia* nigra and to investigate the antibacterial activity of the separated chitosan. Antibacterial activity of both the chitosan were checked against four bacterial strains, namely *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Vibrio cholerae*. The antibacterial activity was measured by disc diffusion method. At the highest concentration of 200 µg/ml, shrimp shell chitosan exhibited zones of inhibition of 4 mm, 5 mm, 6 mm, and 6 mm against *Escherichia coli*, *Vibrio cholerae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* respectively, while *Phallusia nigra* chitosan showed zones of inhibition of 6 mm, 5 mm, 4 mm, and 6 mm against the same bacterial strains. These findings indicate that chitosan derived from both shrimp shells and *Phallusia nigra* exhibits notable antibacterial properties.

Keywords: Chitosan, shrimp shell, *Phallusia* nigra, antibacterial activity etc.

INTRODUCTION

Chitosan is a naturally derived polysaccharide obtained from chitin. Chitin is primarily found in the exoskeletons of crustaceans, insect cuticles, fungal cell walls and certain marine organisms such as ascidians (tunicates). Chitosan is insoluble in water, dissolves in acidic solutions due to protonation of amino groups. It is one of the few natural polycations, allowing it to interact with negatively charged molecules, bacterial membranes, and metal ions. Chitosan is a biodegradable, biocompatible and non-toxic polymer derived from chitin, the second most abundant biopolymer on earth after cellulose. In recent years, alternative sources of chitosan, such as tunicates have been explored due to their chitin-rich tunic structures. Ascidians, or sea squirts, are filter-feeding marine prochordates known for their unique composition, including chitinous tunics that serve as an additional reservoir for chitosan production. Shrimp processing industries generate large amounts of shell waste,

which poses an environmental challenge. These shells are rich in chitin (15-40%), which can be converted into chitosan. The utilization of shrimp shell waste for chitosan extraction aligns with sustainable waste management and provides an economic advantage for commercial applications.

Given the urgent need for eco-friendly, cost-effective, and highly effective antimicrobial agents, chitosan from both shrimp shells and ascidian tunics presents a viable and sustainable solution. Despite extensive research on shrimp-derived chitosan, studies on ascidian-derived chitosan are relatively limited. Additionally, the comparative antibacterial efficacy of chitosan from both sources has not been extensively explored, particularly against clinically relevant human pathogens. This research aims to explore the extraction and antibacterial potential of chitosan derived from these marine sources, assessing its effectiveness against human pathogens responsible for infectious diseases.

MATERIALS AND METHODS

Samples of *Phallusia nigra* Savigny, 1816 were collected from the under surface of the barges of Thoothukudi harbour. Identification upto the species level was carried out based on the key to identification of Indian ascidians by Meenakshi, 1997. Shrimp shell waste materials were collected from Thoothukudi fish market. The samples were washed with sea water to remove sand, mud and then transported to laboratory. The samples were dried and made into powder.

EXTRACTION OF CHITOSAN

Demineralization

The powdered shells were soaked in 1M HCl solution at room temperature for 6-12 hours for the removal of calcium carbonate and other minerals. The mixture was stirred occasionally to ensure uniform demineralization. The suspension was filtered, and the residue was washed with distilled water until the pH became neutral.

Deproteinization:

The demineralized residue was treated with a 3-5% NaOH solution at 80-90°C for 2-4 hours to remove proteins. It was stirred continuously to ensure the complete removal of protein content. The suspension was filtered, and the residue was washed thoroughly with distilled water until the pH became neutral.

Isolation of Chitin:

The residue obtained after deproteinization was chitin, which was the precursor to chitosan. The chitin was dried at 60°C in an oven.

Deacetylation:

The dried chitin was treated with a 40-50% NaOH solution at 100-120°C for 1-4 hours to remove acetyl groups and convert chitin to chitosan. It was stirred periodically during the reaction to ensure uniform deacetylation. The solution was cooled, the chitosan was filtered, and the residue was washed thoroughly with distilled water until the pH became neutral.

Purification:

The chitosan was washed with ethanol to remove residual impurities. It was then dried at 60-70°C until it reached a constant weight. The same procedure was followed for the extraction of *Phallusia nigra* chitosan.

Plate: 1 *Shrimp*

Plate: 2 *Phallusia nigra*

Plate: 3 Chitosan of shrimp shell

Plate: 4 Chitosan of *Phallusia nigra*

Microorganisms

The antibacterial activity of the prepared chitosan from shrimp shell was tested against four bacterial strains, namely *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Vibrio cholerae*. *Staphylococcus aureus* is a Gram-positive bacterium, while *Vibrio cholerae*, *Escherichia coli*, and *Pseudomonas aeruginosa* are Gram-negative bacteria.

Determination of antibacterial activity

Bacterial inoculums were prepared according to the Clinical and Laboratory Standards Institute guidelines. Bacterial cultures were emulsified in normal saline and the

turbidity was adjusted to match the 0.5 Mc Farland turbidity standard. The agar cup method was followed to investigate the antibacterial activity of the extracts. 0.1 mL of TSB broth culture of the test organisms was evenly seeded over the Mueller-Hinton Agar plates. Sterile paper discs of 3 mm were dipped into different concentrations of both chitosan samples (200, 100, and 50 µL). Using forceps, the discs were placed on the MHA plates. The plates were then incubated at 37°C for 24 hours. After the incubation period, the formation of zones around the discs confirmed the antibacterial activity of the respective extracts. Tetracycline antibiotic was used as a positive control.

RESULTS

Antibacterial activity of chitosan prepared from shrimp shells and tunic of marine ascidian *P. nigra* were tested against *Escherichia coli*, *Vibrio cholerae, Pseudomonas aeruginosa*, and *Staphylococcus aureus* shown in Table: 1-6, Figure: 1-6 and Plate: 7, 8.

For *Escherichia coli*, a zone of inhibition of 4 mm was observed at the highest concentration (200 µg/ml) of shrimp shell chitosan, while a 4 mm zone was also noted at 100 µg/ml. No zone of inhibition was observed at 50 µg/ml. In contrast, *Phallusia nigra* chitosan exhibited a maximum zone of inhibition of 6 mm at 200 µg/ml, followed by 5 mm at 100 µg/ml, and 4 mm at 50 µg/ml. For *Vibrio cholerae*, the maximum zone of inhibition (5 mm) was observed at 200 µg/ml for both shrimp shell chitosan and *Phallusia nigra* chitosan. At 100 µg/ml, a 4 mm zone of inhibition was noted, while a 3 mm zone was observed at 50 µg/ml.

For *Pseudomonas aeruginosa*, the shrimp shell chitosan showed a maximum zone of inhibition of 6 mm at 200 µg/ml, followed by 5 mm at 100 µg/ml, and 4 mm at 50 µg/ml. *Phallusia nigra* chitosan exhibited a 4 mm zone of inhibition at 200 µg/ml, 3 mm at 100 µg/ml, and no inhibition at 50 µg/ml.

For *Staphylococcus aureus*, a zone of inhibition of 6 mm was observed at both 200 µg/ml and 100 µg/ml concentrations of shrimp shell chitosan, while a 5 mm zone was noted at 50 µg/ml. Similarly, *Phallusia nigra* chitosan exhibited a 6 mm zone of inhibition at 200 µg/ml, and a 5 mm zone at both 100 µg/ml and 50 µg/ml concentrations.

Figure 1: Antibacterial activity of chitosan from shrimp shell against *Bacterial Pathogens*

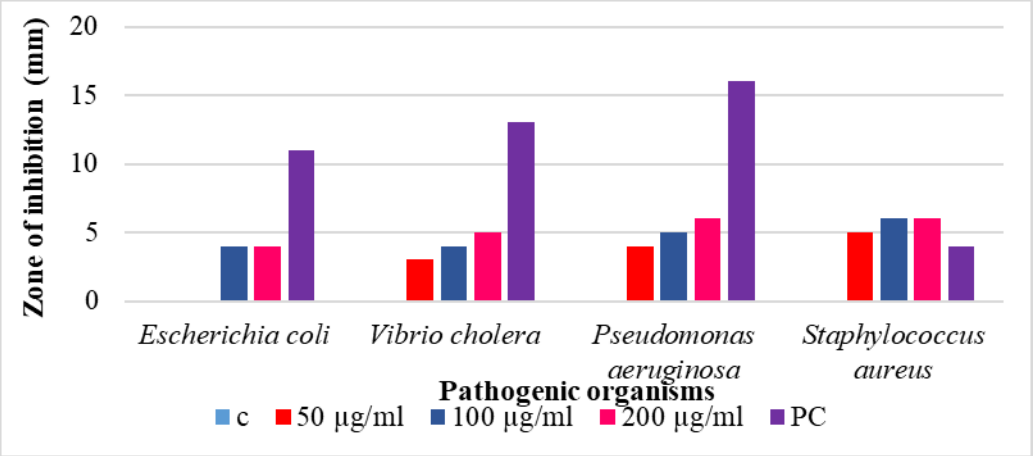


Figure 2: Antibacterial activity of chitosan from *Phallusia nigra* against *Bacterial Pathogens*

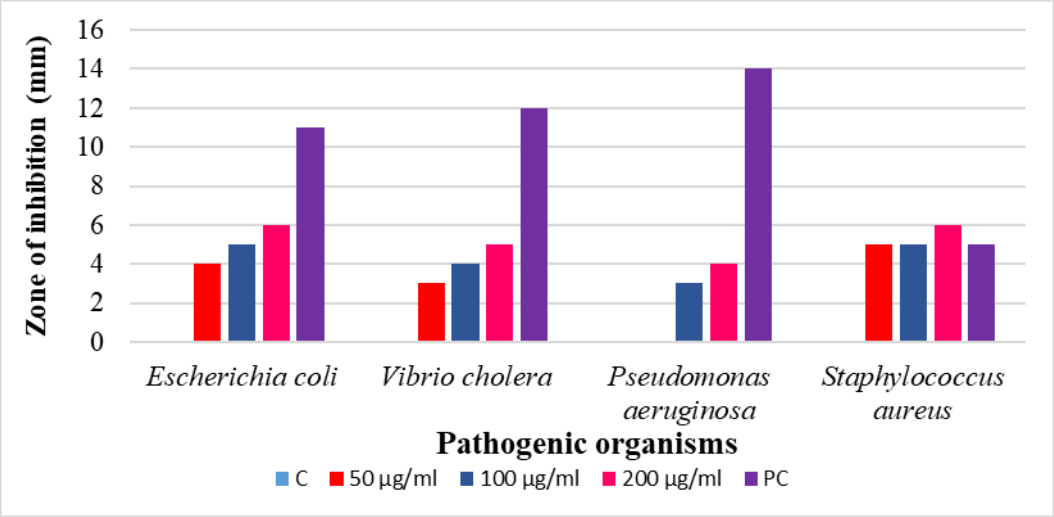


Figure 3: Antibacterial activity of chitosan from shrimp shell and *Phallusia nigra* against *Escherichia coli*

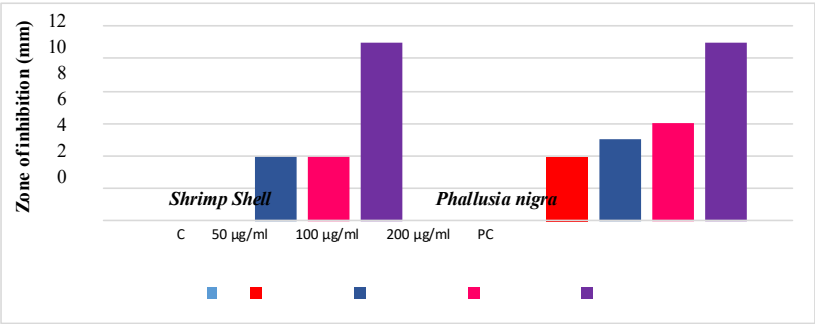


Figure 4: Antibacterial activity of chitosan from shrimp shell and *Phallusia nigra* against *Vibrio cholera*

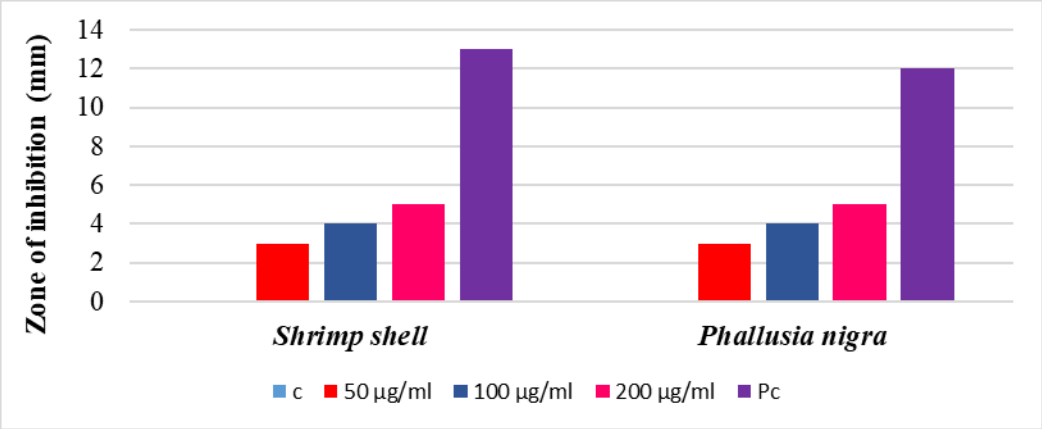


Figure 5: Antibacterial activity of chitosan from shrimp shell and *Phallusia nigra* against *Pseudomonas aeruginosa*

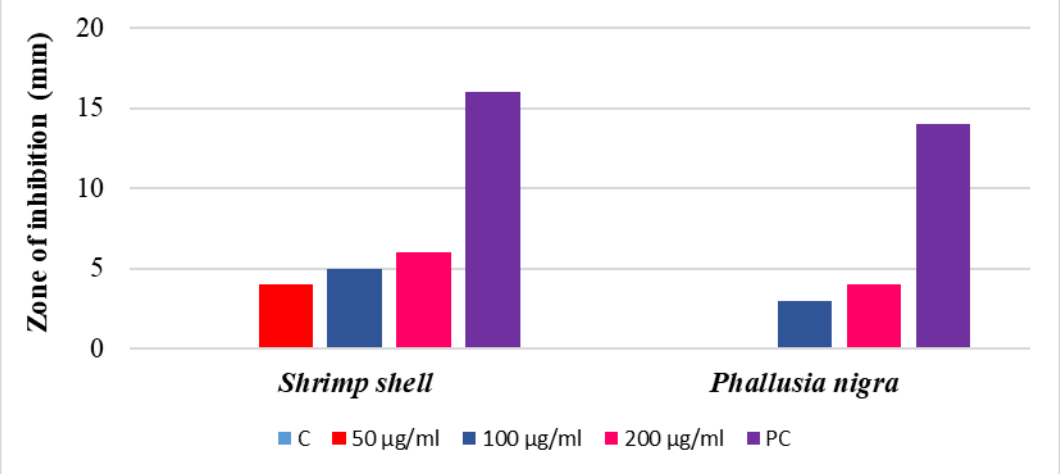
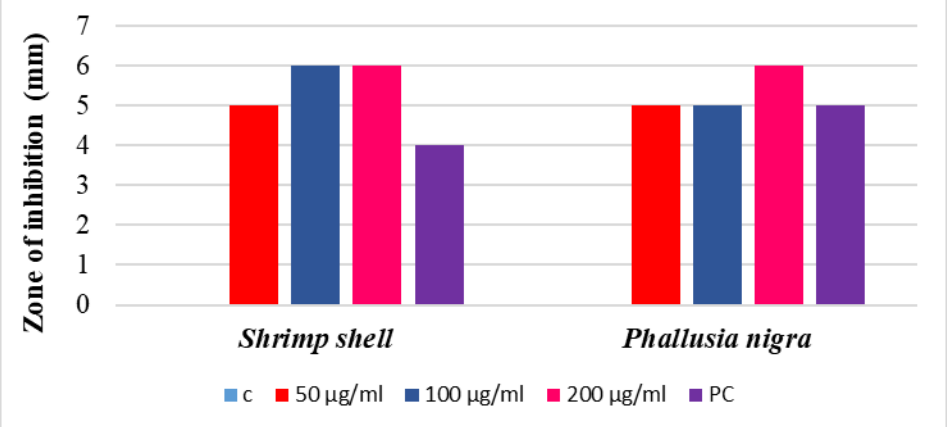
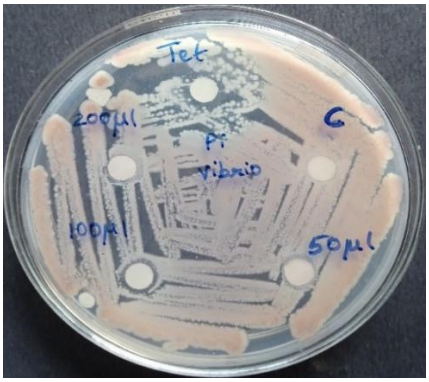


Figure 6: Antibacterial activity of chitosan from shrimp shell and *Phallusia nigra* against *Staphylococcus aureus*



Escherichia coli



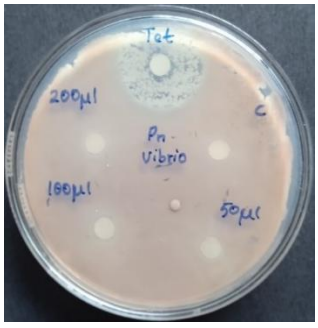
Vibrio cholera

Pseudomonas aeruginosa

Staphylococcus aureus

Plate: 7 Antibacterial activity of chitosan of Shrimp shell against *Bacterial Pathogens*

Escherichia coli



Vibrio cholera

Pseudomonas aeruginosa

Staphylococcus aureus

Plate: 8 Antibacterial activity of chitosan of *Phallusia nigra* against *Bacterial Pathogens*

DISCUSSION

Chitin has also been reported to exhibit a bacteriostatic effect on Gram-negative bacteria, including *Escherichia coli, Vibrio cholerae, Shigella dysenteriae, and Bacteroides fragilis* (Benhabiles *et al.,* 2012).

Chitosan derived from freshwater lobster exhibited notable antibacterial activity, producing inhibition zones of 8.1 mm, 8.2 mm, and 8.8 mm against *Escherichia coli* and zones of 8 mm, 9.2 mm, and 10 mm against *Staphylococcus aureus* (Rani *et al.,* 2023). Additionally, chitosan was found to be 2.2 times more effective than chitin against *Staphylococcus aureus* and 3.0 times more effective against *Escherichia coli* (Tareq *et al.,* 2013).

Crude chitosan from *Phallusia nigra* demonstrated effective inhibition against clinical strains of *Vibrio cholerae* (9.5 mm) and *Vibrio parahaemolyticus* (8.9 mm) at a concentration of 1 mg/mL (Christomelba *et al.,* 2016).

These findings indicate that chitosan from both shrimp shells and *Phallusia nigra* exhibits notable antibacterial properties, with potential applications in biomedical and pharmaceutical fields.

CONCLUSION

The study successfully demonstrated the extraction and characterization of chitosan from both *Phallusia nigra* and shrimp shells, highlighting the superior antibacterial properties of ascidian-derived chitosan. The differences in antibacterial activity are attributed to factors such as the degree of deacetylation, molecular weight, and purity of the chitosan. These findings indicate that marine tunicates can serve as a promising alternative source for chitosan production, contributing to sustainable and eco-friendly applications in healthcare, agriculture, and water treatment. Additionally, the comparative evaluation underscores the potential of ascidian-derived chitosan as a natural antimicrobial agent, providing an effective solution to combat antibiotic-resistant bacteria. Future research focusing on optimizing the extraction process, scaling up production, and conducting in vivo studies will further establish the practical applications of chitosan in various industries.

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