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Research Article

EXTRACTION AND EVALUATION OF ANTIMICROBIAL PROPERTIES OF CHITOSAN DERIVED FROM MARINE CRAB SHELLS

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ABSTRACT

Chitosan has received growing attention since it is biocompatible, biodegradable, and possesses significant antimicrobial potentials. The marine crustacean shell wastes (especially the sea crab shells) are rich sources of chitin and can be utilized as good raw materials in the production of chitosan. This paper is about the process of producing chitosan using shells of sea crabs and assessing its chemical structure and anti-microbial efficiency to determine whether it can be used in industry. Chitosan was obtained using 100 g of dried sea crab shells through a three-step procedure (deproteinization, demineralization, and deacetylation) and was analysed by UV-Vis spectroscopy and FTIR spectroscopy. The antibacterial properties of the samples were evaluated against *Klebsiella pneumoniae*, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli,* and *Salmonella typhi*, whereas antifungal properties tested against *Aspergillus niger, A. flavus* and *A. terreus*. UV -Vis provided the result of strong absorption in wavelength range at 225.0 nm, 275.0 nm and 310.0 nm. The presence of important functional groups such as amine, hydroxyl, nitrile and alkene was confirmed in FTIR spectra, which confirms the successful production of chitosan. The antimicrobial activity was also found to be powerful specifically against *Bacillus cereus* and *Klebsiella pneumoniae*. The Antifungal activity was selective and inhibition was noted significantly on *Aspergillu sterreus.* The retrieved chitosan evidenced positive potential of antimicrobial substance such that sea crab shell waste is a solid source of production of green chitosan. These results facilitate its use as natural antimicrobial agent across industrial sectors. Chitosan isolated using the sea crab shell displayed a broad-spectrum antibacterial activity and a specific antifungal action. Functional structural characterization established effective synthesis of chitosan. These findings underscore the feasibility of its usage as a renewable biopolymer to prevent the growth of microbes.

Keywords: Chitosan, Sea crab shell, Antibacterial activity, Antifungal activity, UV-Vis spectroscopy, FTIR.

INTRODUCTION

Chitosan is a biopolymer, which is abundant, naturally occurring in large quantities and of versatile nature whose candidates are the structural polysaccharide, chitin, which is semi-degraded to chitosan by the removal of acetyl groups. Chitosan has proved to be a desirable biomaterial because of its special physicochemical and biological characteristics as the product of natural materials has been more accepted with the current focus on sustainable materials and environmentally friendly processes. Structurally, chitosan consists of poly-cationic, β (1 4) – linked units of D-glucosamine and N-acetyl-D-glucosamine and has been characterized by high reactivity because of free amino group presence in solution due to solubility in acidic solutions (Kumar, 2000; Rinaudo *et al*.,2006). Sea crab shells are a promising raw material in the extraction of chitosan among the diverse sources, not only based on the high content of chitin but also because its utilization forms part of the valorisation of seafood processing by-products. The general process of the conversion of these bio wastes

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into value-added products, such as chitosan, presents itself as a beneficial practice because the economic and environmental impacts of converting these bio wastes are positive as we are guided by the principles of circular economics and green chemistry (Aranaz *et al*., 2021).Chitosan is used in abundance in an assortment of domains such as biomedical engineering and drug delivery, agriculture and food preservations (Divya *et al*., 2017). Among its most notable characteristics is its antimicrobial quality that characterizes both antibacterial and antifungal properties. It is proposed that the mechanism of action is due to electrostatic forces between the positively charged chitosan molecules and negatively charged microbial cells membranes, which cause membrane disruption and release of cell contents and interfering with the synthesis of DNA/RNA (Kong *et al*., 2010). This has made chitosan a powerful natural antimicrobial additive with prospects of being used as an alternative to artificial chemical preservatives and antibiotics (Goy *et al*., 2009).

Over the past few years, natural antimicrobial agents have gained demand because of increasing concerns on microbial barrier to synthetically produced drugs. Chitosan being biodegradable and non-toxic in nature is an option that is sustainable. Its antimicrobial range has a wide scope in Gram positive and Gram-negative bacteria, pathogen fungi, giving it a prospect of habit use in wound dressing, food coating, medicines, and in pesticides. This paper will focus on the purification of sea crab chitosan by a multi-step chemical method and the characterization of the material in terms of UV-Vis and FTIR spectrum. It also determines its antibacterial and antifungal properties against a selected panel of microbial strains to determine its viability as a natural and sustainable antimicrobial. It addresses the burning issues of waste valorisation and developing microbial control solutions based on biopolymers using an abundant marine by-product.

MATERIALS AND METHODS

Crab Shell Collection

A local fish market in Thiruvananthapuram, Kerala, was used to purchase sea crabs' shells. The shells were then rinsed abundantly in distilled water to wash away the clinging impurities, and allowed to dry in air at room temperature. After drying the shells were ground and powdered with a mechanical grinder. The shell material was crushed into powder and kept in an airtight container until it was to be further processed.

Recovery of Chitosan from Sea Crab Shells

The sequence of chemical steps in the extraction of chitosan was done in three broad procedures, namely: de-proteinization, demineralization and de-acetylation of sea crab shells (Sajina *et al*., 2025). First, 5 g of finely ground crab shells were de-proteinized through the use of 1.25 M sodium hydroxide (NaOH). This mixture was stirred continuously at room temperature, 3 hours to successfully remove the protein content. Upon completion of the reaction, the material was filtered, washed continually with distilled water until a neutral pH was achieved and then air-dried at 100^{o}C in an oven. This dried and de-proteinized sample was then demineralized by incubation at 80^{o}C in 1.25 M hydrochloric acid (HCl) over 5 hours. This enabled the elimination of calcium carbonate and other minerals present in the shells. The chitin obtained was re-filtered, cleaned using distilled water to eliminate any remaining acid, and then dried in a hot air oven. The last process involved the de-acetylation of the purified chitin in order to obtain chitosan. This was done through the treatment of the chitin with 0.5 M NaOH at 100^{o}C over 2 hours. After the treatment, the mixture was filtered and washed with distilled water until the pH was neutral and the mixture was dried. White to off-white chitosan powder was collected as final product that was kept in an airtight container to be used in further analysis.

Characterization of the extracted Chitosan

UV- Visible Spectroscopy

The spectroscopic analysis of the extracted chitosan was done to determine the structural and optical properties of the chitosan (deAlvarenga.,2011). UV-Visible spectrophotometry was used to determine the optical property of the chitosan sample in terms of optical absorption. The absorbance spectrum was measured in room condition (200-400 nm) by Labtronics LT291 spectrophotometer. This test allowed a discussion of the spectral characteristics of the compound and its identification using characteristic absorption bands.

FTIR- Fourier Transform Infrared Spectroscopy

Fourier Transform Infrared (FTIR) spectroscopy was used to further verify the functional groups and molecular structure (Duarte *et al*., 2002). FTIR recording was performed on a Shimadzu instrument which scanned at 4000 to 400 cm $^{-1}$. This method allowed it to detect the presence of functional moieties like amine, hydroxyl and carbonyl groups which are normally found on chitosan.

Scanning Electron Microscopy

The Scanning Electron Microscopy (SEM) was used to analyse at the surface structure of the extracted chitosan. Imaging using scanning electron microscopy (SEM) was done on a high-resolution scanning electron microscope, (ZEISS) at an accelerating voltage of 15 kV. The micrographs were made at multiple magnifications (500x to 5000x) to examine the structural features, such as surface roughness, porosity, and the particle pattern of chitosan matrix (Amaral *et al*., 2005).

Antibacterial Activity of the Chitosan

The agar well diffusion technique was used to determine the antibacterial activity of the extracted chitosan (Johney Jesteena *et al*., 2017). Mueller-Hinton Agar (MHA)

medium was prepared and poured into sterile Petri plates (where about 20 mL of medium was poured into each plate) and solidified under aseptic conditions. The surface of the agar was inoculated with 20 µL of 24 hours old bacterial cultures, such as *Escherichia coli, Staphylococcus aureus, Salmonella typhi*, *Klebsiella pneumoniae*, and *Bacillus cereus* and homogenised using a sterile culture spreader after solidification and plates were left to dry 5 minutes. The agar was punched to create 5 mm diameter wells using a sterilized stainless steel cork borer. All plates were then marked with appropriate bacterial strains names and 20 µL of the chitosan extract added to respective well. The plates were subsequently incubated at 37^0C, over a period of 24 hours. The antibacterial activity was measured after incubation as the diameter of the zone of inhibition around each well, and the effectiveness of chitosan extract on the tested bacterial strains.

Antifungal Activity by agar well diffusion method

To study the antifungal activity malt extract agar was prepared (39gm in 1000ml of distilled water, which was autoclaved and poured to a sterilized petri plate (Johney Jesteena *et al*., 2017). Following solidification 80µl offungal spore suspension of *A.niger*, *A. Terreus*and *A.flavus* was added and spread all over the agar surface using a sterilized cotton swab. The samples were added to the corresponding wells which were made with a sterile cork borer (6 mm in diameter). Then the plates were incubated 3-5 days at 30°C. Fluconazole was taken as a standard positive control. After incubation zone of inhibition was measured in millimeters.

RESULTS AND DISCUSSION

The efficiency of recovering chitosan was 62.22% of the raw material of the dried sea crab shells weight, which amounted to 62.22 g per 100 g of shell weight. This yield reflects the efficiency of the chemical extraction technique used, which is sequential process of de-proteinization, de-mineralization and de-acetylation. Depending on the optimized conditions employed, such as the alkali and acid concentration, temperature, and time of each stage of treatment, the efficiency of this process can be achieved. The yield collected in this article is relatively high compared to the average range of chitosan recovery (41-53%) usually found in different crustacean sources, crab shells, and shrimp (Al Sagheer *et al*.,2009). Even though the current findings were slightly lower than the highest obtained yield of 73.73% of mud crabs as reported (Rosalina *et al*.,2025). it still affirmed the potential of sea crab shells as a source of chitosan that can be utilized as a viable and efficient source of chitosan. These differences in yields could be related to variations in shell contents, species-specific biochemical framework, environmental circumstances, and processing factors. The shells of crustaceans mostly comprise chitin, calcium carbonate, and proteins, but chitin content and mineralization level play a crucial role in the final chitosan output (Tokatlı and Demirdöven.,2018) Sea crab shells, which are omnipresent as seafood processing waste, ensure not only an inexpensive source of raw material but also help achieve environmental sustainability by minimizing marine waste products. This is consistent with the concepts of circular bio economy and valorisation of waste, where the industrial by-products are upgraded into high-value products like biopolymers like chitosan. The economic viability of producing chitosan on a large-scale using crab shells is supported by the high yield that was achieved in this study, especially in areas that have high marine biomass sources located along the coastline.

UV-Visible spectroscopy is also a well-established technique used to characterize the optical and electronic properties of a biomaterial and was successfully used to quantify the properties of chitosan obtained via sea crab shells. This technique offers invaluable data regarding the existence of functional groups, electronic transitions happening in the molecule, and structural integrity that verify the purity and identity of the extracted chitosan. UV-Visible absorption spectrum (Figure 1a) of the chitosan obtained after extracting the shells of the edible sea crab revealed the highest peak at 225 nm, which indicated high absorbance in the UV range. This peak is generally attributable to π -π and core transitions of carbonyls (C=O) within the polymer backbone. Other weak absorbance was noted at around 275 nm and 310 nm, which could be assigned to n to pi transitions involving nonbonding electrons on oxygen and nitrogen atoms on amines and hydroxyl chemical groups. These absorbance bands align with the published literature on the prototypical behavior of chitosan in the spectra (Isa *et al*., 2024; Fajardo). The decreasing pattern of absorbance over 310 nm indicates the absence of any conjugated double bonds or long chromophoric systems, which proves the non-aromaticity and the biopolymer purity. The ability to upload sharp and defined peaks secondly confirms the integrity of the extracted chitosan and the success of the extraction technique used.

The presence of the functional groups in the chitosan found in sea crab shells was confirmed using Fourier Transform Infrared (FTIR) spectroscopy analysis. Because it can identify the exact molecular vibrations of important chemical bonds, this technique finds wide applicability in the structural characterization of polysaccharides, especially chitin and chitosan. Experimental FTIR spectrum (Figure 1 b) of the chitosan sample exhibits a variety of both known absorption bands revealing several specific functional groups characteristic of de-acetylated chitin. A large absorption band at 3443 cm^{-1} arises to the stretching vibrations of -OH and -NH groups, which reflect the hydroxyl and amine frameworks. It also indicates the presence of intermolecular hydrogen bonding by the band at 3204 cm^{-1}, as it is common to chitosan as it has hydroxyl groups and amino groups (Di Santo *et al*., 2020).

The 1658 cm^{-1} band is assigned to amide I (C=O stretching), and the 1559 cm^{-1} band to amide II (N-H bending); both bands verify the partial acetylation of the polymer. Both peaks play an essential role in helping to determine the percentage of de-acetylation (DD), which directly influences the level of solubility and bioactivity of

chitosan. The occurrence of these bands of amide groups reveals that the chitosan still includes some acetyl groups, characteristic of the commercial or semi purified preparations (Raghavankutty *et al*., 2018).It is possible that a small peak at 2246 cm $^{-1}$ could indicate impurities or the expectations of leftover chitin products like incomplete de-acetylation or residual nitrile groups that are more often seen in pure chitosan. The maximum of 1315 cm^{-1} is attributed to -CH bending, and the measured 1056 cm^{-1} is characteristic of C-O-C stretching vibrations and is a key characteristic of the glucosamine backbone. The 827 cm^{-1} band is attributed to C-Cl or C-H out-of-plane bending and may be due to small-scale structural defects, or marine-derived mineral fragments.

In general, the FTIR pattern corresponds to the recognized fingerprint of chitosan, indicating the effective alkali de-acetylation of chitin. The chemical structure of the extracted biopolymer is confirmed by the presence and strength of the amide I and II bands and the presence of the hydroxyl and amino groups. These results are in line with those published in recent studies to identify chitosan and evaluate its purity using FTIR (Venugopal *et al*., 2023). The validity of the structuring as won out by FTIR will be key not just in authenticating the identity of the extracted material, but also in anticipating its performance in use. The identified functional groups directly contribute to the biocompatibility of chitosan, its antimicrobial properties, and its ability to form films, and thus the material can be used in a variety of areas such as medicine, agriculture, packaging, and wastewater treatment. Scanning Electron Microscopy (SEM) was used to analyses the surface morphology of the chitosan obtained on the sea crab shells at 15.0 kV accelerating voltage and magnification compatible with the identification of microstructural features (Figure 1 (c). This topography was very irregular, rough and porous and this can be attributed to chitosan of natural origin, which includes crustacean shells. SEM image revealed many granular formations as well as flake-like structures, and there were areas of scattered shapes that appeared like spheres probably due to residual inorganic content or condensed moisture. Porosity leads to a large surface area, which is an added advantage of their biomedical use, in instance of drug delivery, wound healing, and bacteria films.

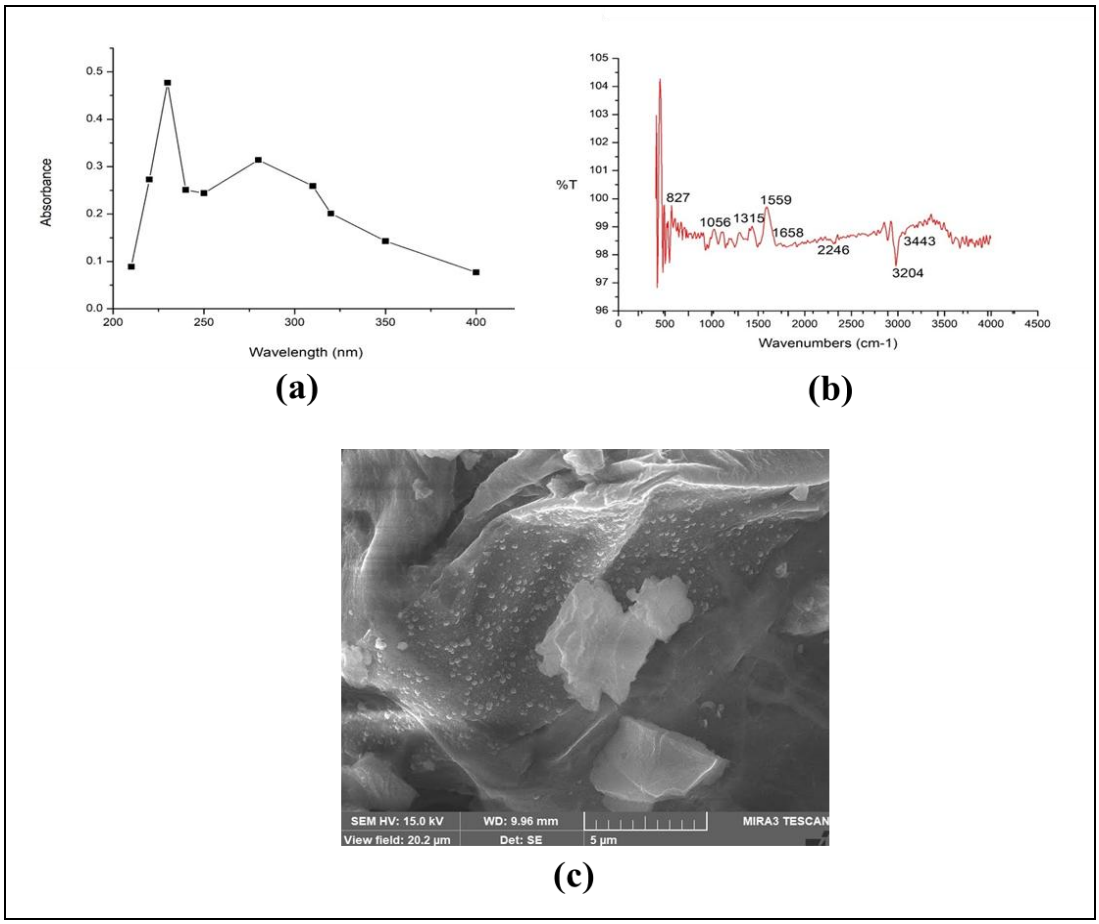

Figure 1. (a) UV- Visible; (b) FTIR spectrum and (c) SEM image of the extracted Chitosan from Crab shell.

Interestingly, the irregular surface texture and particle heterogeneity correspond to earlier published SEM findings of chitosan recovered via de-acetylation of chitin in crab or shrimp shell (Aranaz *et al*., 2009). This clustered structure in the form of flakes implies that it is partially crystalline, and this has a lot to do with deciding what the mechanical strength and solubility of the substance should be. The irregular surface also adds to the characteristics of adsorptive and interaction of chitosan making it apt in the binding of metal ions and anti-microbial action[1].Also, the lack of smooth and glassy areas shows that the extraction was not accompanied by melting or degradation of the polymer, leaving its native bio polymeric characteristics. The microstructure substantiates the argument of effective demineralization and de-proteinization in the extraction process, important in the production of pure chitosan. The biological activity of chitosan, in particular, its antimicrobial activity, is also directly affected by its morphology. It has previously been shown that the rough and porous morphology is capable of facilitating interactions between the microbial cell membrane and the polymer, which leads to significant antibacterial activity (Dutta *et al*., 2004). The antibacterial activity of chitosan of the sea crab shell was tested by agar well diffusion method with five pathogenic bacterial strains which included, *Klebsiella pneumoniae, Bacillus cereus, Staphylococcus aureus, Escherichia coli,* and *Salmonella typhi*. These findings, as shown in Figure 2 indicated a broad-spectrum antibacterial effect with different inhibition zones between bacteria species.Chitosan achieved the greatest antibacterial activity against *Bacillus cereus* (zone of inhibition of 22 mm), *Klebsiella pneumoniae* (21 mm), and *Escherichia coli* (17 mm). Intermediate activity was observed with *Salmonella typhi*, whereas *Staphylococcus aureus* exhibited the lowest susceptibility with a zone of 6mm. Conversely, the blank ($AgNO_3$ solution), had a uniform inhibition zone measurement of 3mm across most bacterial strains suggesting that chitosan was more effective than the control of silver nitrate.

The findings indicate that the chitosan extracted from the shell of the sea crab has strong antibacterial activity, and especially against Gram-negative bacteria, including *K. Pneumoniae* and *E. coli.* It is assumed that the mechanism of action is based on bidirectional electrostatic interactions between chitosan and bacteria that occur between positively charged chitosan amino groups and negatively charged bacterial cell membranes, causing membrane disruption, intracellular content leakage, and cell death (Goy *et al*., 2009; Younes *et al*.,2015). Notably, zones of inhibition have been observed to be greater in Gram-negative strains implying that chitosan extracted can penetrate the outer membrane which is a typical chamber of these bacteria, a feature that increases the antibacterial activity of chitosan. Relatively low activity observed against *S. aureus* on the other hand could be attributed to the difference in cell wall composition and surface charge which hinders the efficient binding of chitosan. These results are in line with the existing studies that chitosan extracted by the marine crustacean source has had high antimicrobial activity, with the activity dependent on the molecular weight, de-acetylation level, and the pH of the medium (Khayrova *et al*.,2023). The viability of the use of sea crab shell waste as a sustainable source of natural antibacterial agents is also supported by the high activity of the sea crab shell waste in this study.

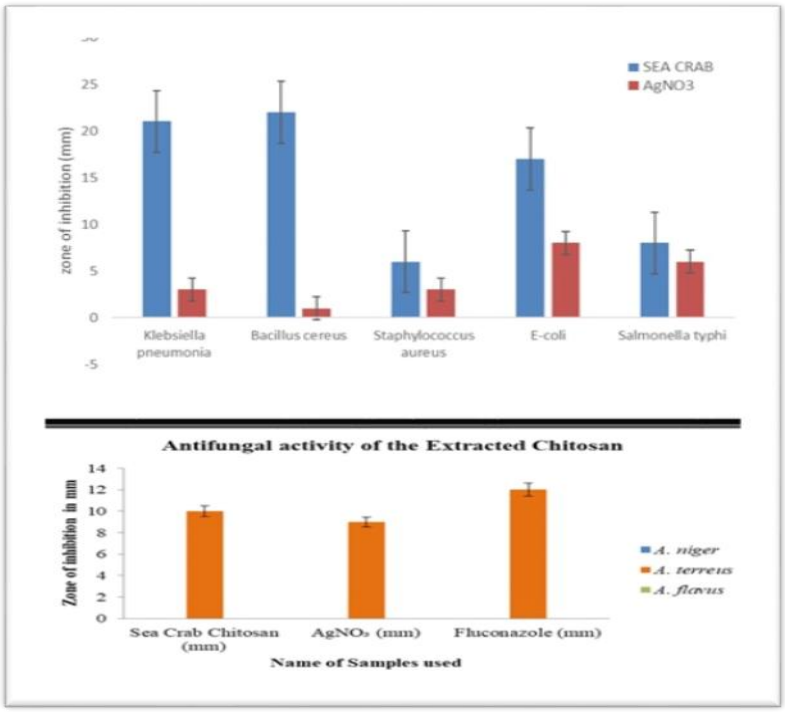

Figure 2. Antimicrobial Activity of the extracted Chitosan.

Agar well diffusion method was used to assess the antifungal effect of chitosan extracted using sea crab shells on three fungal strains, which include *Aspergillus niger, Aspergillus terreus* and *Aspergillus flavus*. The anti-fungal effect of chitosan was compared to that of $AgNO_3$ and fluconazole (F), a commercial anti-fungal drug. Figure 2 summarizes the results. One of the tested funguses, *Aspergillus terreus*, proved to be sensitive to chitosan, but the other two funguses were not recorded to be sensitive to chitosan. The three treatments, however, did not show any antifungal effect against *A. niger* and *A. flavus*, suggesting that the species may have been resistant to the agent or not susceptible under the conditions of the tests.

The antifungal activity against *A. terreus* is also noticeable, and this fungus is an opportunistic agent that can lead to invasive pulmonary aspergillosis, especially in immune compromised people. The antifungal mechanism of chitosan is due to its polycationic nature that enhances its interaction with negatively charged fungal cell walls thereby disrupting cell membrane integrity and causing leakage and death of cells (Abd El-Ghany WA.,2023; Thambiliyagodage*et al*., 2023). Moreover, chitosan is capable of binding to fungal DNA and preventing mRNA and protein synthesis, which also adds to its fungicidal properties (Sahariah *et al*., 2016). The inhibition against *A. niger* and *A. flavus* may be associated with the strong spore coats of these fungi or an increased enzyme resistance that predisposes them less to the chitosan treatment. The comparable strain-specific sensitivity is attributed to some species of *Aspergillus* needed a larger molecular weight or greater levels of de-acetylated chitosan concentration to be inhibited effectively (Badawyand Rabea.,2011). These observations emphasise that chitosan has selective antifungal action and, therefore, its antifungal effect is limited in relation to the fungal species, as well as physicochemical characteristics of chitosan. Even the moderate inhibition of *A. terreus* is a significant implication of the potential of chitosan as a natural, biodegradable, antifungal agent particularly in certain medical or farming applications that might be of fungal contamination**.**

CONCLUSION

This paper supports the viability of seafood wastes, especially sea crab shell wastes, as an effective and sustainable source of raw materials to isolate chitosan-a naturally occurring biopolymer, which was characterized by its biocompatibility, biodegradability, and eco-friendliness. The extraction process applied in the study, one that encompassed de-proteinization, demineralization, and de-acetylation, does not only serve to emphasize a methodical path towards high-quality chitosan recovery, but also contributes to the overall notion of waste valorisation and circular bio economy. The structural determination of UV-Visible spectrophotometry and FTIR spectroscopy, confirm the efficacy of conversion of crab shell chitin to chitosan underscoring the robustness of analytical methods as a means of demonstrating biopolymer synthesis. This systematic transformation of marine trash into a useful biopolymer compliments the contemporary international goals of decreasing environmental influence by means of forming green material. This sustainable development, based on transforming waste so as to create a useful product, contributes to the achievement of sustainable development goals and sets the stage on which the benefits of chitosan can be further exploited in various uses in the industry.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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