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A Comparative Study on the Physicochemical Characterization of Chitosan Obtained from Seafood in Uyo Metropolis, Nigeria

Ugochi E. Ewii[1], Emmanuel O. Olorunsola[2], Adaeze L. Onugwu[3,4], Ojochide M. Ameh[5], Euphemia N. Eke[3], Callistus I. Iheme[1], Anthony A. Attama[3], Chioma Anyiam[1], Toochukwu E. Ogbulie[1]

[1]*Africa Centre of Excellence in Future Energies and Electrochemical Systems, Federal University of Technology, Owerri, Imo State, Nigeria*
[2]*Department of Pharmaceutics and Pharmaceutical Technology, University of Uyo, Uyo, Akwa Ibom State, Nigeria*
[3]*Drug Delivery and Nanomedicines Research Laboratory, Department of Pharmaceutics, University of Nigeria, Nsukka, Enugu State, Nigeria*
[4]*Centre for Research Impact and Outcome, Chitkara College of Pharmacy, Chitkara University, Rajpura, 140401, Punjab, India.*
[5]*Department of Industrial Chemistry, Federal University Dutsin-ma, Katsina State, Nigeria.*

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ABSTRACT

One biopolymer that stands out for its uses in biotechnology and pharmaceuticals is chitosan, whose functionality is often influenced by the source of the material. The primary focus of this study was to derive and analyze chitosan from three different types of seafood shells: crab (*Callinectes amnicola)*, periwinkle (*Tympanotonus fuscatus var. radula)*, and snail (*Archachatina marginata*) collected from Uyo Metropolis, Akwa Ibom State, Nigeria. Chitin was first extracted from the exoskeletons of these organisms and subsequently converted into chitosan using the chemical method. The derived chitosan samples were characterized and compared with commercial chitosan. Yield analysis revealed values of 29.80% for crab, 16.31% for periwinkle, and 39.80% for snail. All samples had a degree of deacetylation above 50%. Fourier Transform Infrared Spectroscopy (FTIR) analysis showed consistent features, including O-H stretching vibrations, N-H amine groups, alkyl peaks, and glycosidic bonds characteristic of chitosan. Despite chemical similarities, physical differences were observed. Snail chitosan had high thermal stability but low crystallinity, whereas crab and periwinkle chitosan samples showed moderate crystallinity. The commercial chitosan was highly crystalline. Ash content was highest in periwinkle-derived chitosan (10.46%). While all chitosan samples demonstrated comparable solubility, viscosity varied. These findings indicate that while the chitosan samples share core chemical structures, source-specific differences in physical properties may influence functional applications. The study concludes that seafood waste provides a viable source of functional chitosan, with varying characteristics influencing their potential uses, especially in pharmaceutical science and technology.

Keywords: Chitosan, Snails, Periwinkles, Crabs, Characterization, Seafood waste

Introduction

Uyo, the capital city of Akwa Ibom State in Southern Nigeria, is renowned for its abundant seafood, particularly in its local markets. This makes it a practical and sustainable source of shell waste for chitosan production. The most commonly consumed seafood includes snails (*Archachatina marginata*), crabs (*Callinectes amnicola),* shrimps (*Penaeus monodon*), oysters (*Crassostrea gigas*), crayfish (*Procambarus clarkii*), and periwinkles (*Tympanotonus fuscatus var. radula*).[1] Most of these foods have protective shells covering their fleshy parts. A schematic view of some of these shells is shown in Figure 1.[2-4] One important component of these shells is chitin. The Greek word "chiton," meaning "covering," is where the term "chitin" originates. Chitin is a naturally abundant polysaccharide and is regarded as the second most common biopolymer in nature, following cellulose. In addition to crustacean shells, chitin is present in the exoskeletons of insects, molds, bacteria, and fungi.[5]

*****Corresponding author. E mail: ugochiyereatuchukwu@yahoo.com
Tel: 08135362049

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The beta glycosidic linkages (in the 1,4 position) bind the units of 2-acetylamino-2-deoxy-D-glucose that comprise its chemical structure.[6,7] There are two different processes for producing chitin: the chemical approach and the biological method. The biological approach is less expensive and better for the environment.[8] This includes the enzymatic method (utilizing enzymes such as alcalase, trypsin, pepsin, and papain) and the fermentation method (involving microbes like *Pseudomonas aeruginosa, Bacillus subtilis*, and *Lactobacillus plantarum*).[9] Three processes are involved in the manufacture of chitin using the chemical method: demineralization, deproteinization, and decolorization.[9,10] Other methods that can be employed include ultrasound extraction,[11] microwave-assisted extraction,[12] and utilizing ionic liquids and deep eutectic solvents. Deacetylation transforms chitin into chitosan, a linear polymer consisting of randomly distributed beta-linked D-glucosamine and N-acetyl-D-glucosamine.[13] Figure 2 presents a schematic illustration of the chitosan structure.[14] Known to be biocompatible, non-toxic, biodegradable, and eco-friendly, the applications of chitosan are extensive, including drug and gene delivery, wound dressing, tissue engineering, bioimaging, and regeneration of bone tissues. It is a thickener, preservative, water purifier, and antibacterial in the food sector. In agriculture, chitosan inhibits fungal, bacterial, and viral growth, making it an effective antimicrobial agent in treating plant diseases. In industry, chitosan may be included in producing hair colorants, shampoos, hair sprays, creams, lipsticks, toothpaste, mouthwashes, and more.[15] Since chitosan's physicochemical properties are influenced by its source and method of preparation, evaluating chitosan derived from less commonly studied shells like periwinkles,

snails, and crabs becomes essential to understanding their suitability for pharmaceutical applications. [16] For instance, chitosan derived from crustaceans has a higher deacetylation degree, molecular weight, and even greater impurities than chitosan obtained from fungi.[17] Additionally, the acid treatment during extraction affects the total yield of chitosan. Heated sulfuric acid yields less chitosan than lactic acid, while hydrochloric acid produces chitosan with a better extent of deacetylation than formic and acetic acid.[18] Higher concentrations of acid used during treatment result in darker chitosan with an enhanced degree of deacetylation. Moreover, it is well recognized that the degree of deacetylation significantly determines chitosan's mechanical, physicochemical, and biological characteristics. It also influences its solubility. For example, chitosan with a low extent of deacetylation (>60%) has low solubility in polar solvents,[19] while chitosan possessing a high extent of deacetylation (70-85%) is partly soluble, and chitosan characterized by a very high degree of deacetylation (85-95%) is highly soluble in polar solvents. Interestingly, these varying properties of chitosan influence its applicability. The level of deacetylation, for example, impacts chitosan's bacterial activity. More deacetylated chitosan inhibited *P. aeruginosa* and *E. coli* because it contained amino groups.[20] Chitosan, having a higher molecular weight (>300kDA) and extent of deacetylation (70-80%) is suitable for drug delivery systems, tissue engineering, and food packaging, while chitosan with a lower deacetylation degree (55-70%) and lower molecular weight (<300kDA) is better suited for gene delivery and plant protection. Thus, the source of chitosan, its extent of deacetylation, and other properties are crucial in determining its applicability.[21]

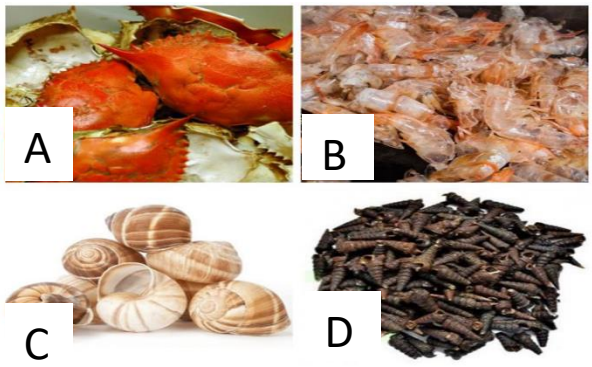


Figure 1: Photographs of seafood shells used in chitosan extraction: (A) Crabs *(Callinectes amnicola*), (B) Shrimps (*Penaeus monodon*), (C) Snails (*Archachatina marginata*), and (D) Periwinkles (*Tympanotonus fuscatus*)[2-4]

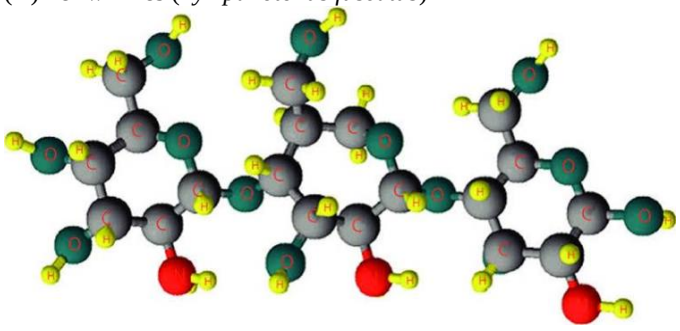


Figure 2: Schematic representation of chitosan's structure

In light of these considerations, the study was designed to employ a chemical method for the extraction and characterization of chitosan from the shells of three seafood types—crabs (*Callinectes amnicola*), periwinkles (*Tympanotonus fuscatus var. radula*), and snails (*Archachatina marginata*) sourced from Uyo metropolis, Nigeria. The chemical method was chosen due to its efficiency, easy availability of reagents, minimal time consumption, and lack of requirement for specialized equipment making it suitable for large-scale applications. The extracted chitosan samples were compared with commercial chitosan in terms of physicochemical properties, including degree of deacetylation, solubility, viscosity, thermal stability, crystallinity, moisture content, ash content, surface morphology, and chemical structure. These properties were evaluated using Differential Scanning Calorimetry (DSC), Gas Chromatography-Mass Spectrometry (GC-MS), Fourier Transform Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD), and Scanning Electron Microscopy (SEM).[22] The study aimed to assess the suitability of locally sourced seafood shells for chitosan production by extracting and characterizing the biopolymer and comparing its properties with those of commercial chitosan using standardized analytical techniques. Additionally, this work serves as a foundation for future functional investigations, such as antimicrobial efficacy and biocompatibility testing, to fully establish chitosan's therapeutic and industrial potential derived from locally available marine sources. These studies are essential for validating the use of indigenous chitosan as a viable and sustainable alternative to imported commercial variants in pharmaceutical and industrial applications in Nigeria and beyond. While previous studies have investigated the extraction and characterization of chitosan from conventional sources such as shrimp and crab,[23,24] there is limited information on chitosan derived from less commonly explored seafood waste, like snail and periwinkle.[25,26] This study is distinct in its focus on these alternative local sources, offering a comparative physicochemical analysis alongside commercial chitosan using a range of analytical techniques to identify sustainable, low-cost materials for biomedical applications.

Materials and Methods

Materials

Commercial chitosan powder (degree of deacetylation: 75%) was purchased from the ADELS® scientific store in Lagos State, Nigeria. Waste shells of periwinkle (*Tympanotonus fuscatus var. radula*), snails (*Archachatina marginata*), and crabs (*Callinectes amnicola* and related species) were collected from local markets in the Uyo metropolis (5° 2' 20.2668" N and 7° 54' 34.0920" E). Analytical-grade reagents, including sodium hydroxide (≥98% purity), hydrochloric acid (37%), distilled water, and deionized water, were obtained from Sigma-Aldrich in St. Louis, MO, USA.

Methods

Extraction of chitosan from crustaceans

Chitosan extract was obtained from shells of periwinkles, snails, and crabs using the chemical method, i.e., the four-step procedure (homogenization, deproteinization, demineralization, and deacetylation) adopted by Eke-Ejiofor & Moses (2019).[27]

Homogenization

Snail, crab, and periwinkle shells were gathered, cleaned with clean water, sun-dried for two days, then oven-dried for forty-eight hours at 150°C, and then ground into fine powder using a mechanical grinder (BSC series Seashell Crusher, Brightsail Machinery Co., Ltd., Jiangsu, China). A sealed container was used to keep the ground shells.

Deproteinization

The milled shells were deproteinized by boiling them in 3.5% aqueous sodium hydroxide for three hours at 100°C while stirring constantly. The solid-to-solvent ratio was 1:10 (w/v). The alkaline was drained from the shell, cleaned to neutrality with distilled water, and desiccated at 90°C.

Demineralization

A water bath was used to heat the deproteinized samples for five hours at a higher temperature after mixing them with 1.25 N HCl. Calcium carbonate is eliminated in this way. After draining off the acid, the residue was rinsed thoroughly with distilled water to remove residual acid.

Deacetylation

The removal of acetyl groups from the chitin molecule and the transformation of chitin into chitosan are known as deacetylation

processes. This was done by treating the chitin with aqueous sodium hydroxide (NaOH) at 40 to 50% (w/v) for seven hours at 90°C to deacetylate it. Following deacetylation, the alkali was drained off and cleaned using deionized water. Ultimately, the chitosan was oven-dried for one hour at 105°C to ensure complete moisture removal.

Percentage yield of chitosan

The percentage yield of chitosan was derived by measuring the weights of the ground shells and the resultant chitosan following each extraction.[27] Equation 1 was utilized to ascertain the percentage yield of the samples.

$$\text{Yield (\%)} = \frac{\text{Weight of chitosan sample}}{\text{Weight of the ground shells}} \times 100\% \quad \text{.....(1)}$$

Determination of the degree of deacetylation

The procedure used by Adekanmi *et al.* (2023) was utilized with a few modifications.[28] At room temperature for 1 hour, 0.1g of the chitosan sample was dissolved in 25 mL of hydrochloric acid (0.06 molarity). Subsequently, the solution was diluted to 50 mL with purified water, and titration was performed using sodium hydroxide at a concentration of 0.1 N until frequent shaking was observed to achieve a pH of 3.75. The volume of sodium hydroxide was measured and recorded at this pH. The total volume of sodium hydroxide (0.1M) was noted at the end of the continuous titration process when the pH reached 8. Equation 2 was used to estimate the deacetylation degree[29].

$$\text{DDA (\%)} = 2.03 \times \frac{V2 - V1}{m + 0.0042\,.(v2 - v1)} \quad \text{..... (2)}$$

Where m is the weight of the chitosan sample
V2 is the volume of NaOH at pH 8
V1 is the volume of NaOH at pH 3.75
0.0042 is the coefficient that results from the difference in molecular weights of chitosan and chitin monomer units
2.03 is the coefficient that results from the molecular weight of the chitin monomer unit

GC-MS analysis

GC-MS analysis was conducted using the BUCK M910 gas chromatograph equipped with an HP-5MS column (30 m × 0.25mm × 0.25µm film thickness) and a mass spectrometer (Buck Scientific Inc., East Norwalk, CT, USA). This was done to detect and quantify the bioactive compounds and impurities found in the chitosan samples. Electrons (that were high in energy, 70 eV) were utilized (inside an electron ionization device) for spectroscopic detection by the GC-MS. The carrier gas utilized was pure helium gas (99.99%). Its rate of flow was 1ml/min. The temperature was first set at 50°C, raised by 3°C every minute, and maintained for 10 minutes before reaching 300°C. A splitless injection of one microliter of the produced 1% extracts diluted with acetonitrile was made. Based on the peak area generated in the chromatogram, the relative amounts of the chemical components found in each of the chitosan extracts were expressed as a percentage. Compound identification was based on GC retention times and comparison of mass spectra with standard databases (Replib and Mainlib) in the GC-MS system.[30]

FTIR spectroscopy

Key functional groups of the chitosan samples were determined using an FTIR spectrometer (Nicolet Nexus 670, Thermo Fisher Scientific, Madison, WI, USA), as demonstrated by Atuchukwu *et al.* (2021).[31] Chitosan samples were ground with spectroscopic grade potassium bromide (KBr) (1 mg of dried sample and 100 mg of dry KBr) and scanned over a frequency range of 3500 to 1000 cm^{1} after being desiccated in a vacuum oven at 60°C overnight.

Scanning electron microscopy (SEM)

A single scanning electron microscope (Hitachi TM1000, Tokyo, Japan) running at an accelerated voltage of 15 kilovolts was used to determine the samples' morphologies. The microscope had an energy dispersive spectroscopy (EDS) fitted. Samples were prepped for SEM by placing them on carbon tape with sputter coating.[32] Images were taken at 500 – 2000× magnification with scale bars included.

Differential scanning calorimetry (DSC)

Using the nitrogen atmosphere method outlined by Earnest *et al.* (2022), DSC analysis was performed using a Discovery Differential Scanning Calorimeter (TA Instruments, New Castle, DE, USA). Eight milligrams of the samples were placed in aluminum pans and heated at a rate of 10°C/min from ambient temperature to 300 °C.[33]

X-ray diffraction (XRD)

A Siemens D5000 X-ray Diffractometer (Siemens AG, Karlsruhe, Germany) equipped with CuKα radiation (λ = 1.5406Å) was used to record the X-ray diffraction patterns of the samples, in reflection mode over an angular range of 5 to 60 ° (2Θ) at 40 kV, 30mA, and room temperature. This was done to determine the crystalline structure of the chitosan samples.[34]

Ash content determination

The ash content of the chitosan samples was determined using a high-temperature muffle furnace (SX2-4-10A, Zhongxing Laboratory Equipment Co., Ltd, Henan, China) operated at 530°C for 20 hours using Orji *et al's* method.[35] A high-temperature muffle furnace was utilized. The ash content of the chitosan samples was calculated using Equation 3 below.

$$\text{Ash content (\%)} = \frac{\text{Total weight of ash}}{\text{Total weight of sample}} \times 100 \quad \text{.....(3)}$$

Determination of moisture content

Gravimetric techniques were used to determine the moisture content of chitosan.[36] For two hours, the chitosan samples were kept at a constant weight in an air-heated oven (Memert, Germany). By comparing the weights of the wet and dry samples in the oven, the moisture percentage was determined. Equation 4 was utilized to ascertain the moisture content.[37]

$$\text{Moisture Content (\%)} = \frac{\text{Wet weight} - \text{dry weight}}{\text{Sample weight}} \times 100 \quad \text{.....(4)}$$

Determination of viscosity

The method utilized by Adekanmi *et al* was used with modifications.[25] A Brookfield digital viscometer (Model DV-E, Brookfield Engineering Laboratories, Middleboro, USA) was used to measure the viscosity of chitosan that had been extracted from shells at 25°C and 50 rpm. Measurements were conducted using a 1% (w/v) chitosan solution in 1% acetic acid.

Determination of solubility

The solubility of the chitosan samples was determined following a modified method described by Adekanmi *et al.* (2023).[25] Briefly, 0.1 g of each chitosan sample (W_0) was dissolved in 10 mL of 1% (v/v) acetic acid and stirred continuously at room temperature for 60 minutes. The resulting solution was centrifuged at 10,000 rpm for 15 minutes to separate the undissolved fraction. The supernatant was carefully decanted, evaporated, and dried at 60°C to a constant weight (W_1). The solubility (%) of the chitosan was calculated using Equation 5. All measurements were performed in triplicate (n = 3), and results were expressed as mean ± standard deviation.

$$\text{Solubility (\%)} = \frac{W0}{W1} \times 100 \quad \text{..... (5)}$$

W0 = Initial weight of the chitosan sample (before dissolution) (g)
W1 = Weight of the dissolved chitosan (supernatant) after centrifugation and drying (g)

Chitosan dissolution in solvents

The ability of the chitosan samples to dissolve in different solvents was ascertained by placing 10g of each sample in 100 mL of several solvents (water, chloroform, petroleum ether, methanol, ethanol, ethyl acetate, etc.) at room temperature.

Data and statistical analysis

All experimental measurements were made in triplicate (n=3), and the results were reported as mean ± standard deviation. Using SPSS version 25 (IBM Corp., USA), one-way analysis of variance (ANOVA) was used to determine whether there were any significant differences between the chitosan samples. Differences were deemed statistically significant when $p < 0.05$.

Results and Discussion

Percentage yield of chitosan

The percentage yield of chitosan from crab shells, periwinkle shells, and snail shells was 29.8%, 16.31%, and 39.8%, respectively. The percentage yield of chitosan from crab shells (29.8%) could be compared to the yield of chitosan from crab shells by Sreeja *et al* (21%),[38] Mulatsari *et al* (18.1%),[39] and Rimadhanti *et al* (32.2%).[40] This high yield could be attributed to the high chitin found in crab shells. Several species of crabs are found in the Uyo metropolis. These include *Callinectes amnicola, Panopeus africanus, Cardisoma armatum and Goniopsis pelii.*[41] These species have a high chitin content and thus yield a large quantity of chitosan.[42,43] Another factor that could be attributed to the high yield of chitosan is the efficient extraction method, as suitable concentrations of acid and bases were utilized at optimum conditions. The percentage yield of chitosan from periwinkle shells (16.31%) could be attributed to the low chitin content derived from the major species of periwinkles found in Uyo, Akwa Ibom State, Nigeria (*Tympanotonus fuscatus var. radula* and *Pachymelania aurora*).[44] Other species of periwinkles are known to yield higher chitosan content, e.g., *Littorina punctata* (46.37%) and *Nodolittorina natalensis* (35.85%).[45] The percentage yield of chitosan from snails (39.8%) could be compared to the yield of chitosan from the study done by Adekanmi *et al* (44.73%).[25] This high yield may be ascribed to the high chitin content in the snail species predominant in Uyo, Akwa Ibom State (*Archachatina marginata* and *Archachatina archachatina*).[25] The abundance of these seafoods justifies the use of extracted chitosan as an economic source of revenue.

The degrees of deacetylation

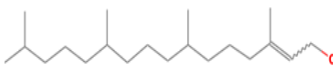
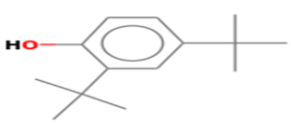
The deacetylation degrees of commercial chitosan and chitosan from shells of crabs, periwinkles, and snails were deduced to be 75.0%, 86.6%, 71.0%, and 62.5%, respectively. The level of deacetylation of chitosan refers to the number of acetyl groups in chitin replaced by hydrogen atoms to create chitosan. It represents the proportion of N-acetyl-D-glucosamine units converted to D-glucosamine units in the polymer. This parameter is important in determining the solubility as well as the biological activity (e.g., biodegradability and biocompatibility) of the polymer. A surge in the DDA of chitosan will cause a rise in the solubility of the polymer (especially in polar solvents) due to the heightened presence of free amino groups, which enhances hydrogen bonding with water molecules.[46] A degree of deacetylation that is greater than or equal to 50% is suitable for chitosan.[47] Thus, all the samples have adequate degrees of deacetylation. The variations in the degrees of chitosan could be ascribed to the different sources of chitin, extraction methods, and the analytical methods used to ascertain the results.[48] Also, the high DDA of periwinkle-derived chitosan suggests a greater potential for applications requiring highly soluble polymers, such as injectable hydrogels and transdermal drug delivery systems.

GC-MS analysis

Although chitosan is a high-molecular-weight polymer and not volatile, GC-MS was applied to the chitosan extracts to identify any low-molecular-weight, volatile compounds present. These compounds may have originated from residual lipids, fatty acids, or other bioactive molecules retained during the extraction process. The presence of such compounds can contribute to the biological activity and functionality of chitosan, particularly in pharmaceutical or biomedical applications. The retention time (RT), compound names, structure, molecular weight, and biological activities of the major components identified in the chitosan samples are summarized in Tables 1-4. The GC-MS chromatograms of all the analyzed samples are seen in Figures 3a-d.

In the GC-MS chromatogram of chitosan from snail shells, two major compounds are predominant: oleic acid and 2-Methyl-Z, Z-3,13-octadecadienol. The presence of these compounds may be due to the composition of snail shells and the process of extraction. Found in snails and other marine organisms, oleic acid is often found in the lipid fraction of these shells. Its presence may be due to its incomplete removal during the extraction process.[49] Though less common, 2-Methyl-Z, Z-3,13-octadecadienol may also be found in the lipid component of snail shells.[50] Nevertheless, these compounds are associated with suitable biological activities as seen in Table 1. Oleic acid is known to enhance the percutaneous absorption of many drugs,[51] while 2-Methyl-Z, Z-3,13-octadecadienol is known to have antimicrobial and anti-inflammatory activity.[52,53]

Table 1: Retention times (Rt), compound names, molecular weights, and biological activities of the major components identified in snail-derived chitosan by GC-MS analysis

Retention Time (Rt)	Compound Name	% Composition	Structure	Molecular Weight	Biological Activities
5.388	1-Hexadecanol, 3,7,11,15-tetramethyl-	1.23		298.547	Anticancer, anti-inflammatory, antioxidant, and antimicrobial
12.096	Dodecane	1.89		170.335	Antibacterial activity and antifungal activity
13.507	(-)-Carvone	9.36		150.218	Antidiabetic, anti-inflammatory, anticancer, neurological, antimicrobial, antiparasitic, antiarthritic, anticonvulsant
21.166	2,4-Di-tert-butylphenol	0.46		206.324	Strong antifungal and antioxidant
34.762	Oleic acid	18.95		282.461	Percutaneous absorption enhancer of several drugs, as it introduces the disruption of the lipid structure of the

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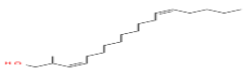
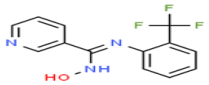
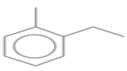
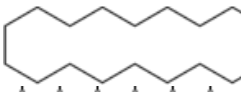
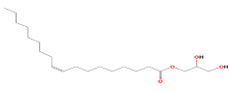
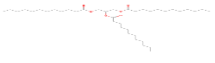
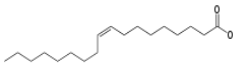
					stratum corneum, allowing drug permeation
36.089	2-Methyl-Z,Z-3,13-octadecadienol	17.77		280.488	Antimicrobial agent, anti-inflammatory agent, Anticancer agent, Cosmetic and dermatological application
36.259	Pyridine-3-carboxamide, oxime, N-(2-trifluoromethylphenyl)-	7.17		281.233	Anticancer agent, antimicrobial agent (Tentative identification possibly due to instrumental artifact or contamination).

In the chromatogram of chitosan obtained from periwinkle shells, three compounds are dominant. These include oleic acid, dodecanoic acid 1,2,3-propanetriyl ester, and 9-octadecenoic acid (z)-2, 3-dihydroxy propyl ester, as seen in Table 2. The presence of these compounds may be due to the fatty acid content of periwinkle shells, the method of chitosan extraction, contamination of the periwinkle shells, and artifacts during the GC-MS analysis. These compounds have previously been associated with antimicrobial and wound-healing activities in the literature, although further bioactivity studies are required to confirm these effects in the extracted chitosan samples.[54] However, their presence in chitosan samples may also reflect incomplete purification, and their biological contributions should be interpreted with caution. The presence of cyclopropane,1,2-dimethyl-3-methylene in chitosan from crab shells is also due to its chemical composition, the process of extraction, and even the presence of contaminants. As seen in Table 3, these compounds have anti-cancer and anti-microbial activity.[55,56] The commercial chitosan was observed to contain squalene, 9-octadecanoic acid (Z)-2,3-dihydroxypropyl ester, and 13-octadecenal (as seen in Table 4), even though 9-octadecanoic acid (Z)-2,3-dihydroxypropyl ester and 13-octadecenal are often lipid residual impurities, which are often present due to insufficient purification.[57] Periwinkle and crab chitosan however, showed a greater potential for promoting the healing of wounds with the presence of bioactive compounds like 9-octadecenoic acid (Z)-2,3-dihydroxypropyl ester (10.13%, 3.72% concentration respectively), Dodecanoic acid-1,2,3-propanetriyl ester (17.25% concentration) present in the periwinkle chitosan and 9-ethyl- (28.37% concentration), Hexadecenoic acid, methyl ester (0.90% concentration), 9-octadecenoic acid (Z)-methyl ester (2.96% concentration), 9-octadecenoic acid (Z)-2,3-dihydroxypropyl ester (3.72% concentration) found in the crab chitosan.

Overall, GC-MS analysis revealed that chitosan extracted from different seafood sources retained various bioactive molecules, which may enhance their suitability for specific therapeutic applications such as wound healing, drug delivery, or antimicrobial formulations. However, further bioactivity studies may be required to evaluate their actual contributions to these applications. Compounds with unclear origins may be due to contamination or instrument artifacts.

Table 2: Retention times (Rt), compound names, molecular weights, and biological activities of major components identified in periwinkle-derived chitosan by GC-MS analysis

Retention time (rt)	Compound name	% Concentration	Structure	Molecular weight	Biological activities
5.486	Benzene, 1-ethyl-2-methyl-	1.56		120.192	Toxic and irritant properties, could impede wound healing and pose health risks.
21.230	Tetracosane	1.28		366.707	Skin Protectants and Emollients, Lubricants, and for coating
22.754	Hexadecane	3.27		226.445	Nanoparticle formulation, Solvent carrier, and emollient
31.771	1-Docosene	4.93		308.585	Penetration enhancer, Emollient in skincare
32.214	9-Octadecenoic acid (Z)-2,3-dihydroxypropyl ester	10.13		356.540	Emollient and moisturizer, Anti-inflammatory agent, antimicrobial agent, promotes wound healing, source of linoleic acid, Transdermal Delivery, Carrier for active ingredients
33.604	Dodecanoic acid, 1,2,3-propanetriyl ester	17.25		639.001	Antibacterial, Antiviral, and antifungal agents, emollients, and moisturizers. Promotes the healing of wounds, Carrier of active ingredient
34.751	Oleic acid	19.00		282.460	Percutaneous absorption enhancer. Drugs can permeate the corneum by disrupting the lipid structure.

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36.655	9-Octadecenoic acid (Z)- 2,3-dihydroxypropyl ester	16.03		356.540	Emulsifying agents, Moisturizers, and emollient, antimicrobial agents, promote wound healing.

Table 3: Retention times (Rt), compound names, structures, molecular weights, and biological activities of major components identified in crab-derived chitosan by GC-MS analysis

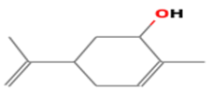
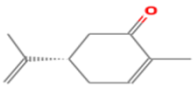
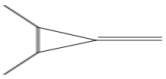
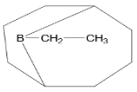
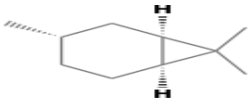
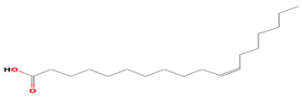
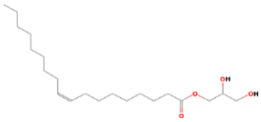
Retention time (Rt)	Compound name	% concentration	Structure	Molecular weight	Biological activities
13.400	Carveol	3.36		152.233	Anti-nociceptive, Anti-inflammatory agent, antimicrobial agent, Inhibits cancer, Anti-anxiety.
13.507	D-Carvone	5.70		150.210	Anti-nociceptive, Anti-inflammatory agent, antimicrobial agent, anti-bacterial, Inhibits cancer, Anti-anxiety.
13.590	Cyclopropane,1,2-dimethyl-3-methylene	17.01		82.150	Synthetic application, antimicrobial activity, Flavour and fragrance
13.622	9-Borabicyclo[3.3.1]nonane, 9-ethyl-	28.37		150.100	This has been reported in some studies to show biological activity, but its therapeutic potential in chitosan remains speculative.
14.841	Bicyclo[4.1.0]heptane,3,7,7-trimethyl-(1α,3α,6α)-	0.48		138.250	Anti-inflammatory, anti-oxidant, antimicrobial agent, analgesic, bronchodilator effects, Anti-anxiety, Anti-cancer, neuro-protective
29.538	Hexadecanoic acid, methyl ester	0.90		270.451	Anti-inflammatory agent, and antimicrobial agent, Inhibits cancer, and wound healing.
31.157	9-Octadecenoic acid (Z)-, methyl ester	2.96		296.488	Anti-inflammatory agent, antimicrobial agent, emollient, penetration enhancer, anti-oxidant, and wound healing.
33.744	Cis-Vaccenic acid	4.59		282.461	Anti-Atherogenic Effects, Lipid profile improvement, Anti-inflammation, Insulin sensitivity, anticancer, antimicrobial
33.939	Oleic acid	1.75		282.461	It enhances the percutaneous absorption of some medications, as it induces the disruption of the stratum corneum's lipid structure, allowing the permeation of drugs
36.056	9-Octadecenoic acid(Z)-,2,3-dihydroxypropyl ester	3.72		356.540	Emulsifying agents, Moisturizers and emollients, and antimicrobial agents. It may promote wound healing.

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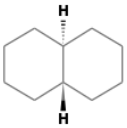
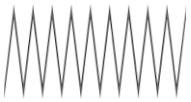
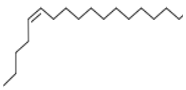
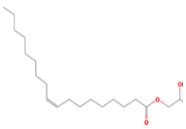
Table 4: Retention times (Rt), compound names, molecular weights, and biological activities of the major components identified in commercial chitosan by GC-MS analysis

Retention time	Compound name	% Concentration	Structure	Molecular weight	Biological activity
7.815	Naphthalene,decahydro-, trans-	2.05		138.250	Industrial solvent, Microbial degradation
7.940	Decane, 1-fluoro-	1.12		160.272	Hydrophobic, alters absorption.
26.21	Eicosane	1.71		282.547	Carrier for hydrophobic drugs, emollient, enhance cell membrane interaction
26.639	2-Methylhexacosane	1.39		380.733	Protective coating, microbial interaction
32.704	13-Octadecenal	16.63		266.462	Antimicrobial agent
34.173	1-Hexacosene	1.35		364.691	Antimicrobial, hydrophobic
34.683	9-Octadecenoic acid (Z)-,2,3-dihydroxypropyl ester	17.62		356.540	Emollient and moisturizing properties, anti-inflammatory and anti-microbial agent
36.276	Squalene	21.14		410.718	Moisturizing agent, anti-aging, anti-oxidant, anti-inflammatory agent, immune system support, anti-cancer, regulates cholesterol.

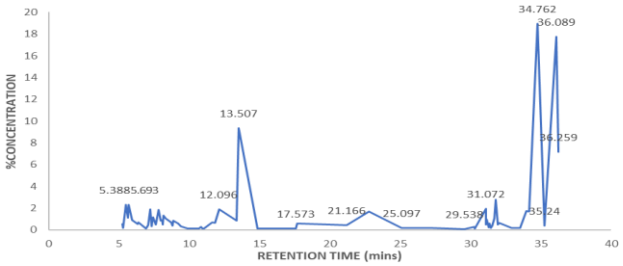


Figure 3a: GC-MS chromatogram of snail-derived chitosan

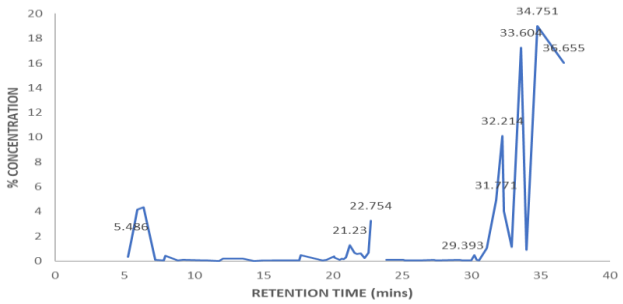


Figure 3b: GC-MS chromatogram of periwinkle-derived chitosan

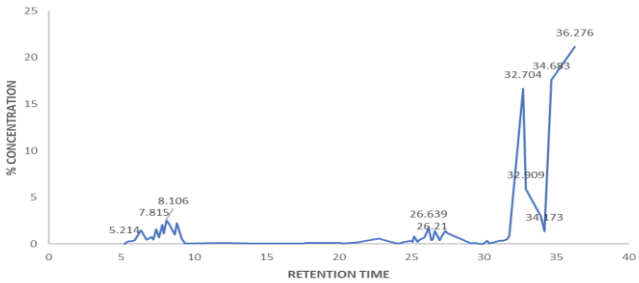


Figure 3c: GC-MS chromatogram of commercially obtained chitosan sample

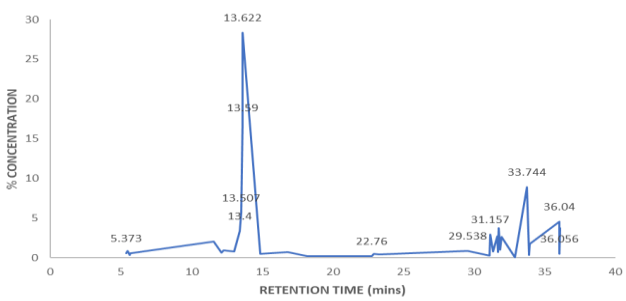


Figure 3d: GC-MS chromatogram of crab-derived chitosan sample

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FTIR analysis

The FTIR spectra of the chitosan samples from shells of cabs, snails, periwinkles, and the commercial chitosan are shown in Figures 4a-d. Similar FTIR spectra are observed for all the chitosan samples. The peaks at 3254.89, 3557.91, 3519.56, and 3224.79 cm-1 are typically indicative of the stretching vibration of O-H (hydroxyl groups). Peaks at 2915.32, 2971.76, 2958.47, and 2940.42 cm-1 are typical for alkyl groups present in the polysaccharide structure of chitosan. FTIR spectra of all the extracted and commercial chitosan samples revealed the presence of primary and secondary amine groups, indicating successful deacetylation. This observation aligns with findings by Ghannam *et al.*[58] Peaks at 1634.08, 1611.64, 1622.18, and 1600.11 cm-1 are typical of the amide group; the presence of amide I and II bands indicate residual acetyl groups from chitin and the characteristic structure of chitosan. Peaks at 1180.41, 1274.93, and 1246.04 cm-1 for snail, periwinkle, and crab are the strong peaks important for the glycosidic bonds that form the backbone of the chitosan polymer. The variations in the FTIR spectra highlight differences in the purity and extent of deacetylation of the chitosan samples from the different sources. Higher-purity chitosan, with fewer impurities and a higher degree of deacetylation, is generally preferred for pharmaceutical applications and wound healing, due to its enhanced biocompatibility and bioactivity. The commercial chitosan and the one derived from snails may be more suitable for drug formulation owing to their higher purity levels however, comprehensive testing of drug-loading capacity, release profiles, and cytotoxicity is necessary before confirming such suitability. Also, unique bioactive molecules in crab and periwinkle-derived chitosan could offer additional therapeutic benefits.

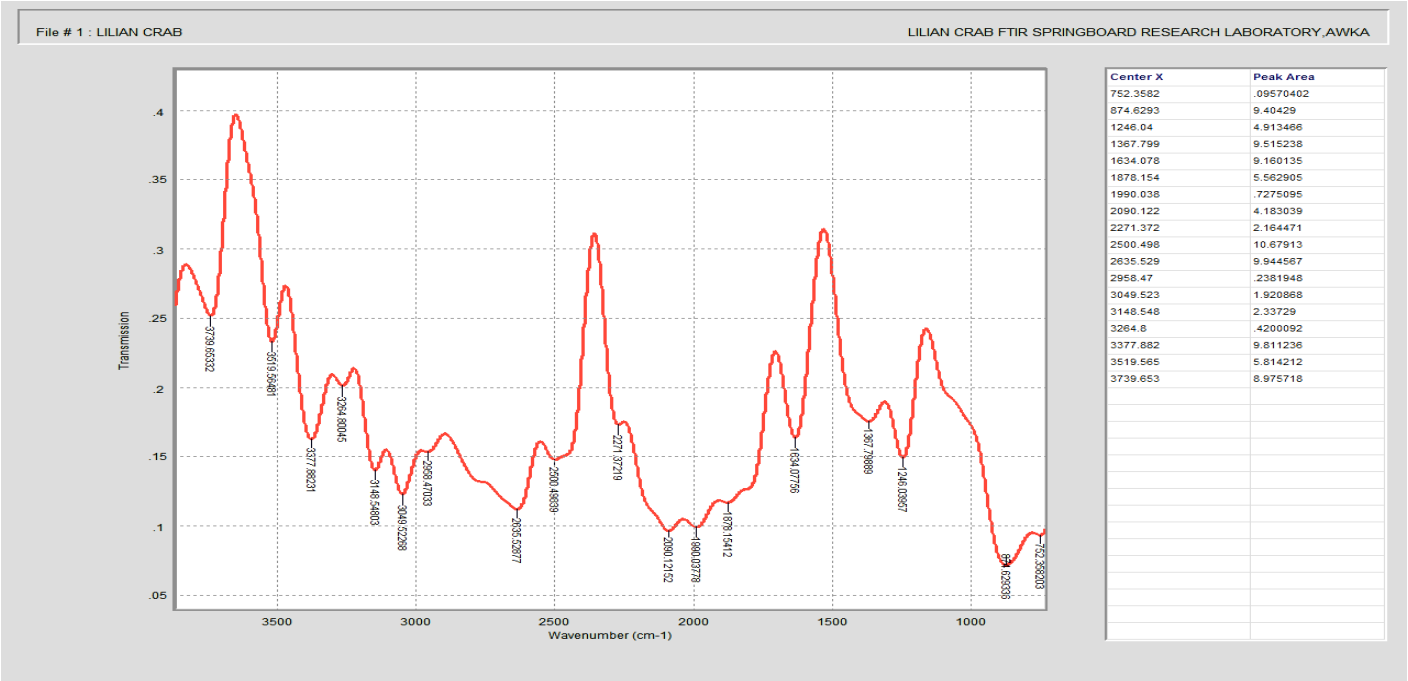

Figure 4a: FTIR spectra comparing functional group vibrations of crab-derived chitosan

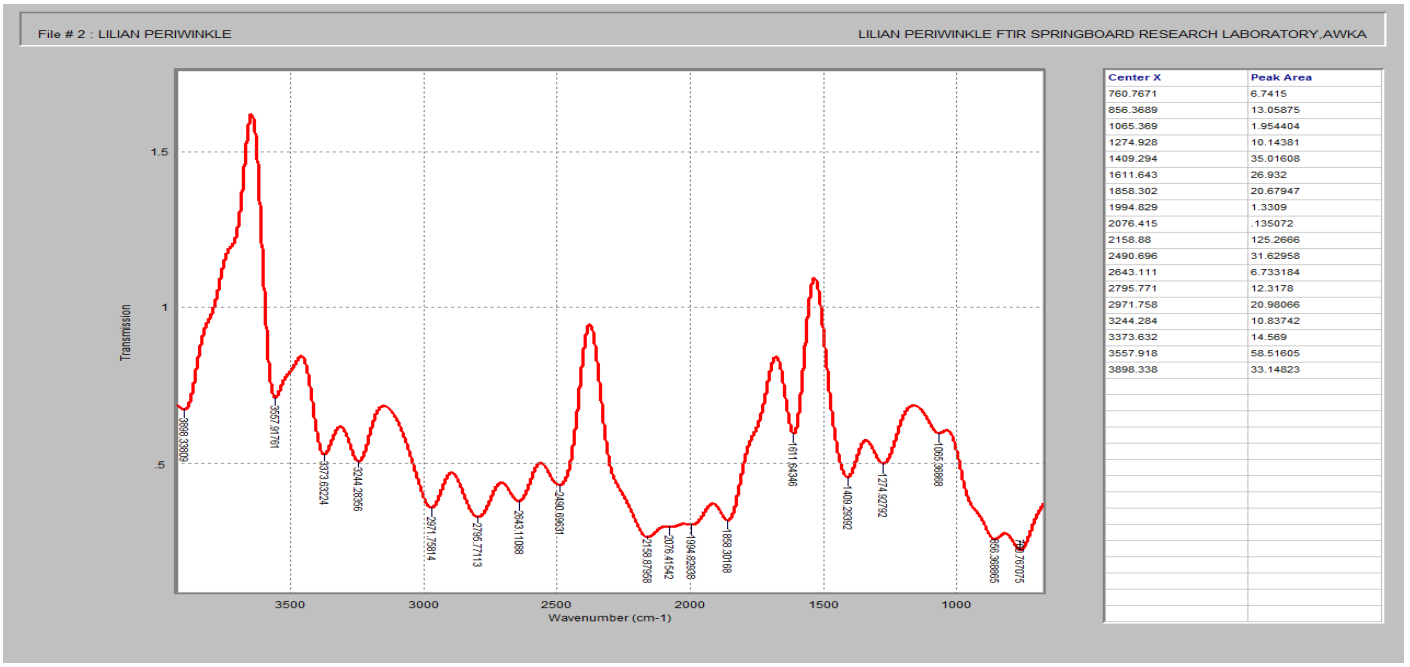

Figure 4b: FTIR spectra comparing functional group vibrations of periwinkle-derived chitosan

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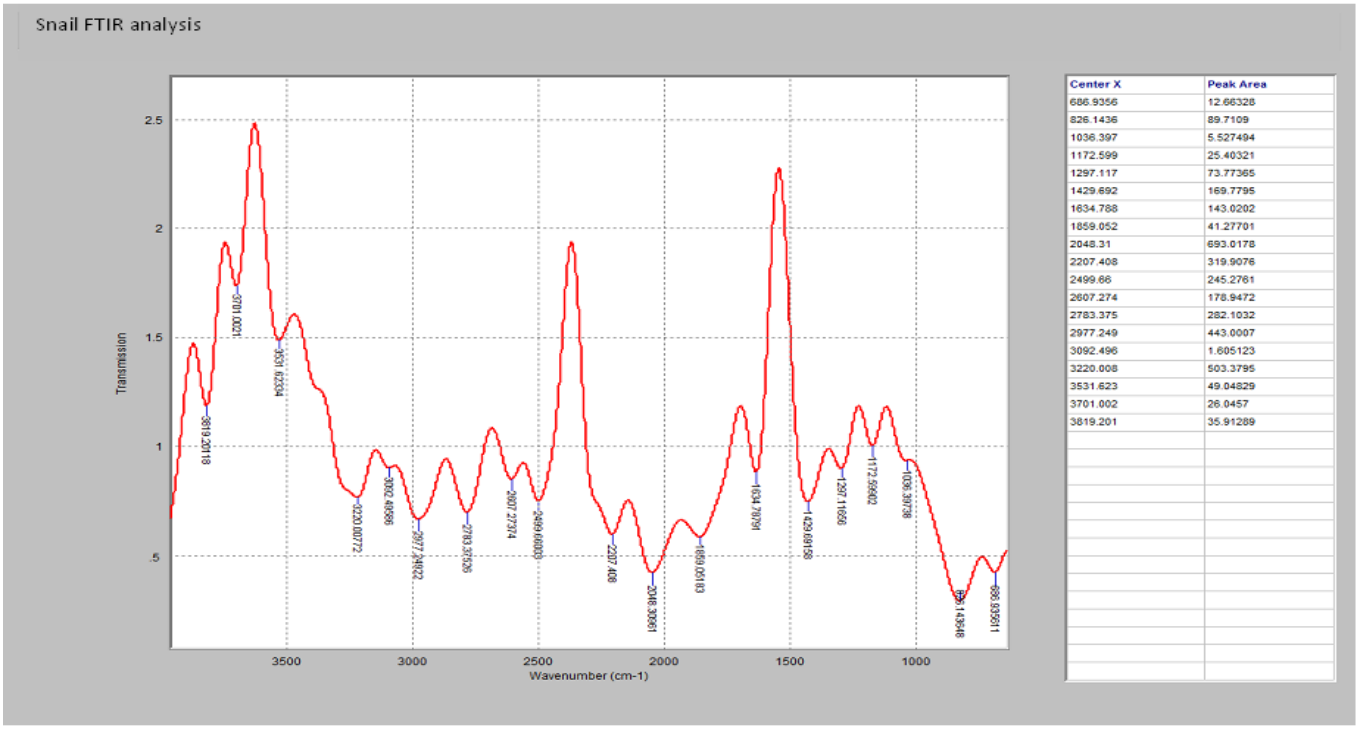
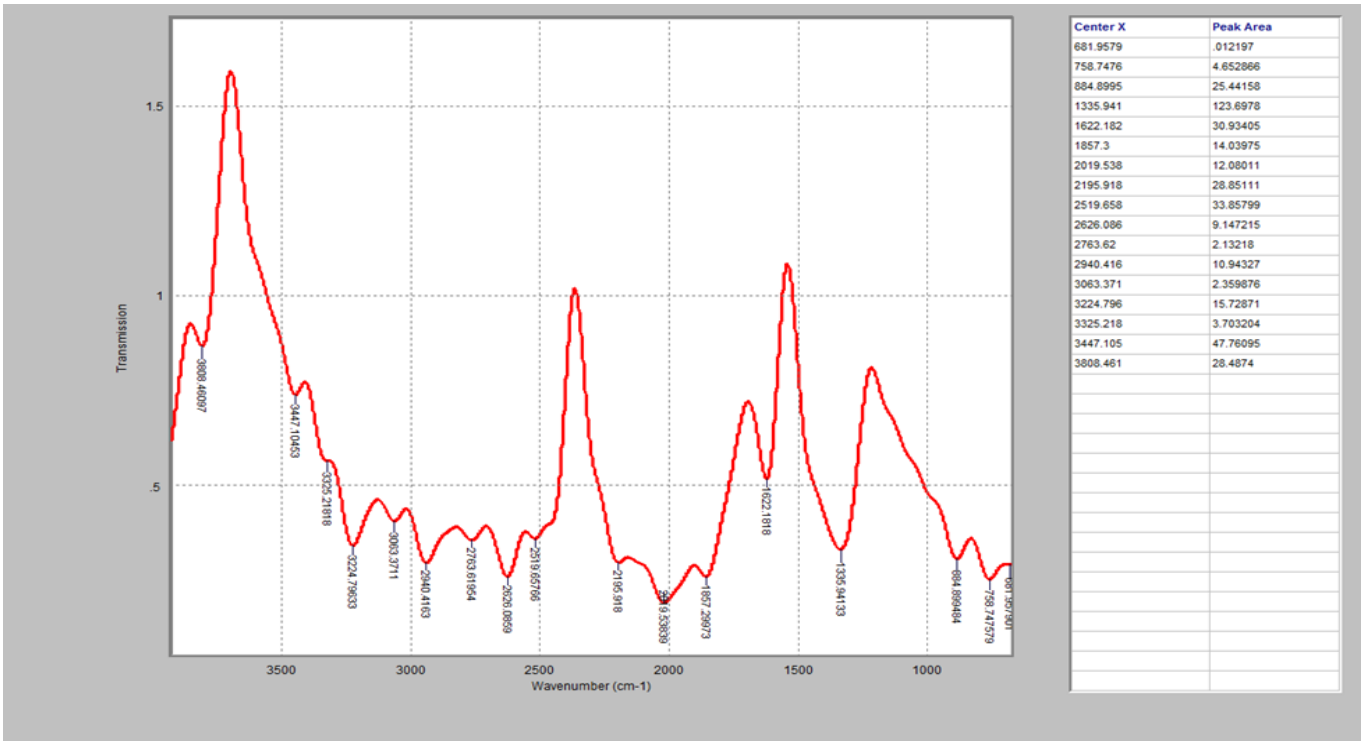


Center X	Peak Area
686.9356	12.66328
826.1436	89.7109
1036.397	5.527494
1172.599	25.40321
1297.117	73.77365
1429.692	169.7795
1634.788	143.0202
1859.052	41.27701
2048.31	693.0178
2207.408	319.9076
2499.66	245.2761
2607.274	178.9472
2783.375	282.1032
2977.249	443.0007
3092.496	1.605123
3220.008	503.3795
3531.623	49.04829
3701.002	26.0457
3819.201	35.91289

Figure 4c: FTIR spectra comparing functional group vibrations of snail-derived chitosan



Center X	Peak Area
681.9579	.012197
758.7476	4.652866
884.8995	25.44158
1335.941	123.6978
1622.182	30.93405
1857.3	14.03975
2019.538	12.08011
2195.918	28.85111
2519.658	33.85799
2626.086	9.147215
2763.62	2.13218
2940.416	10.94327
3063.371	2.359876
3224.796	15.72871
3325.218	3.703204
3447.105	47.76095
3808.461	28.4874

Figure 4d: FTIR spectra comparing functional group vibrations of commercially-obtained chitosan

SEM analysis

The SEM images of chitosan obtained from periwinkles, snails, and crabs are revealed in Figures 5a-d. This analysis revealed the morphologies of the powders. Crab-derived chitosan exhibited a smooth, homogeneous surface with moderate porosity. Chitosan derived from periwinkle shells revealed irregular shapes with a rough texture and higher porosity. Snail chitosan was observed to have an irregular particle shape, polygonal structure, and higher porosity. The commercial chitosan's small particle size and high homogeneity may indicate excellent coverage and consistent performance in wound healing, drug delivery, and more applications. However, snail chitosan's irregular particle shape, polygonal structure, higher porosities, and significant inhomogeneities may require further refinement to ensure uniform application and effectiveness for wound healing and several applications, as also observed by Tertsegha *et al.*[59]

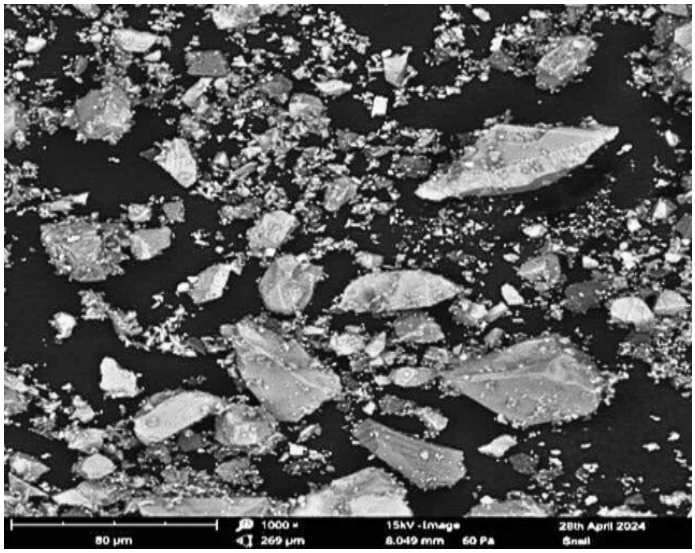


Figure 5a: SEM image of chitosan sample derived from snails

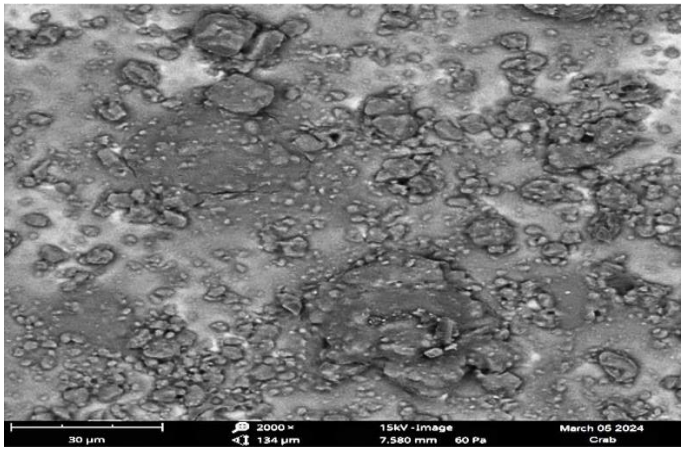


Figure 5b: SEM image of chitosan sample derived from crabs

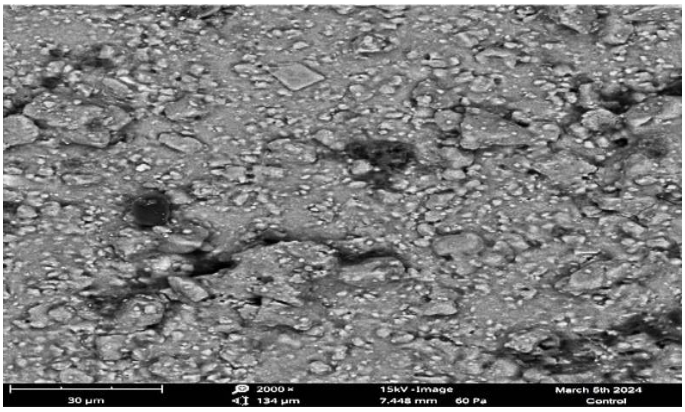


Figure 5c: SEM image of the commercial chitosan

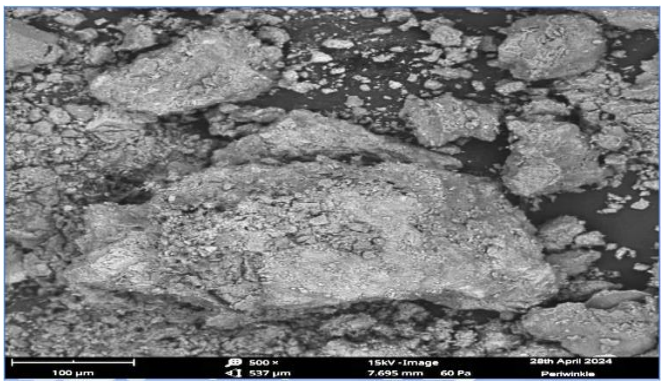


Figure 5d: SEM image of chitosan derived from periwinkles

DSC analysis

Thermal stability, as determined by DSC, showed that snail-derived chitosan had superior stability compared to the others, as seen in its broader endothermic peak and delayed thermal transition (Figures 6a-d). High thermal stability indicates a highly ordered internal structure. This suggests that snail chitosan may be more appropriate for applications that involve sterilization at high temperatures, such as implantable medical devices. The moderate thermal stability of crab and periwinkle-derived chitosan suggests that these forms are more appropriate for applications where thermal degradation must be minimized, such as low-temperature drug formulations.

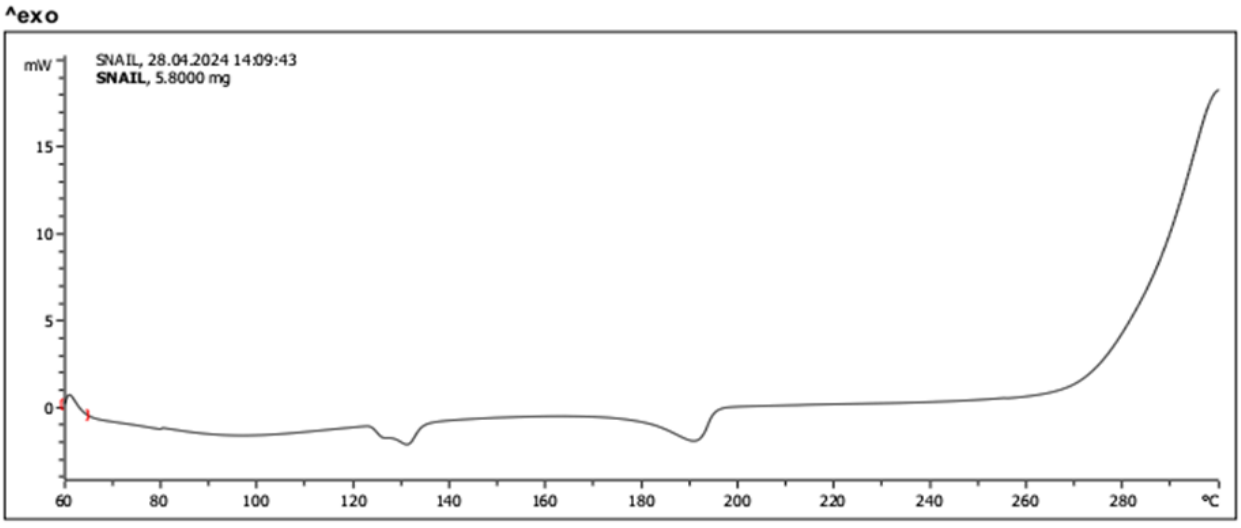


Figure 6a: DSC thermogram showing the thermal behavior of snail-derived chitosan

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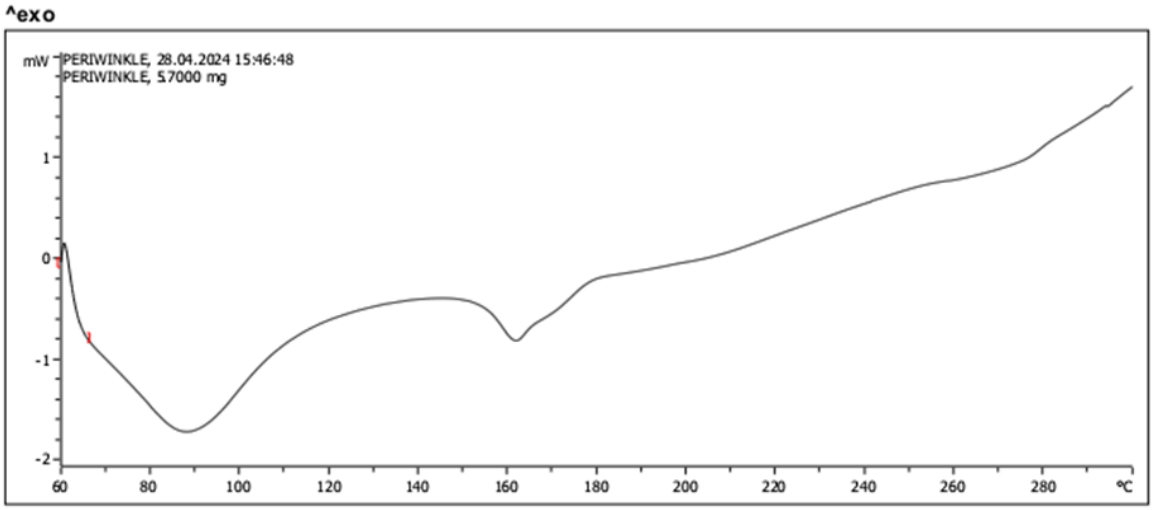

Figure 6b: DSC thermogram showing the thermal behavior of periwinkle-derived chitosan

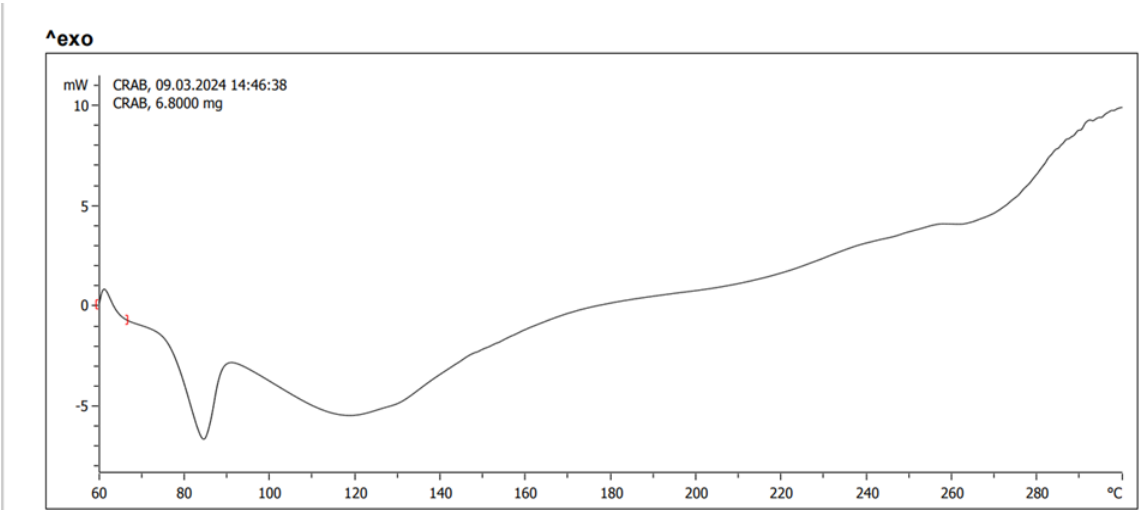

Figure 6c: DSC thermogram showing the thermal behavior of crab-derived chitosan

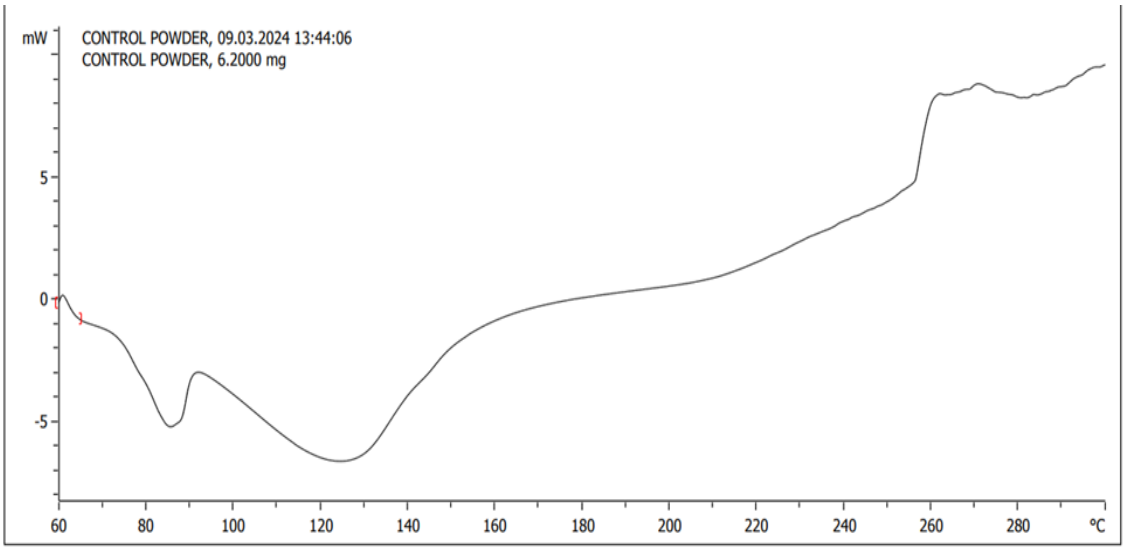

Figure 6d: DSC thermogram showing the thermal behavior of the commercial chitosan

XRD patterns

The XRD patterns of the commercial chitosan and the chitosan derived from shells of crabs, periwinkles, and snails are shown in Figures 7a-d. This analysis reveals significant differences in crystallinity, phase composition, and structural properties, which are crucial for their use in wound recovery and other pharmaceutical uses. High crystallinity in

chitosan has been linked to slower degradation rates and prolonged mechanical strength.[60] Periwinkle and crab chitosan exhibit moderate and well-ordered crystalline structures. The peaks observed in the XRD pattern at 2θ values between 12^0 and 29^0 provide a balance of mechanical strength and flexibility. Snail chitosan, with its lower crystallinity and higher peaks observed in the XRD pattern at 2θ values between 27^0 and 53^0, offers enhanced solubility and faster biodegradation. The commercial chitosan with peaks observed in the X-ray diffraction (XRD) pattern at 2θ values between 12^0 and 26^0 indicates high crystallinity. This suggests potential suitability for controlled-release applications, although further studies on drug-release kinetics are necessary. The combination of peaks across different angles indicates that the chitosan might offer a controlled release profile for the incorporated drug, with variations in crystallinity affecting how the drug diffuses from the matrix.

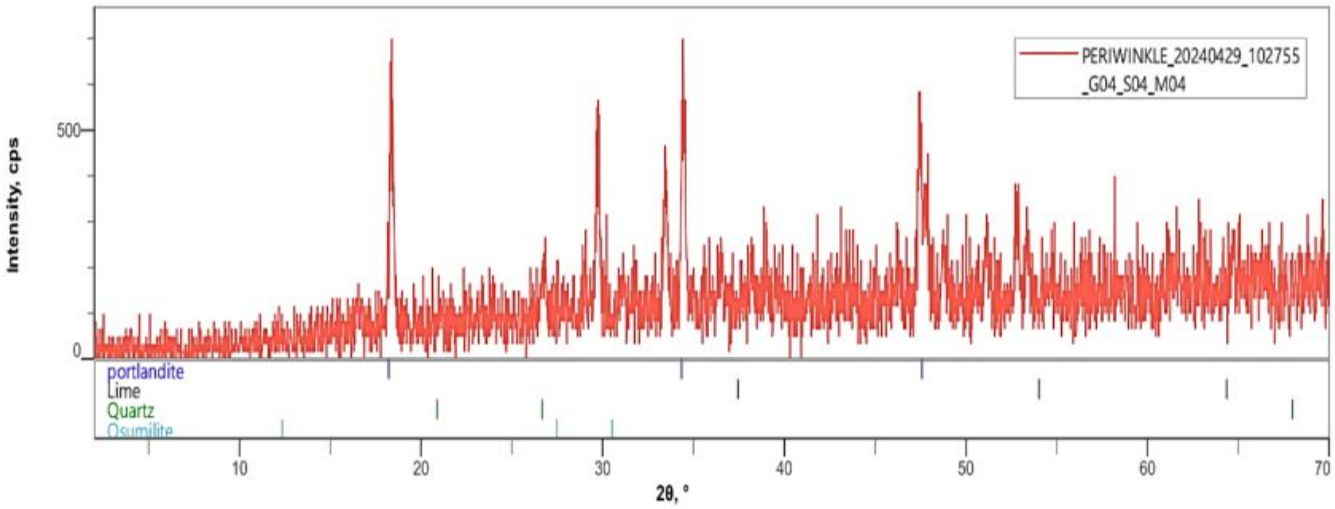

Figure 7a: XRD pattern illustrating the crystalline structure of periwinkle-derived chitosan

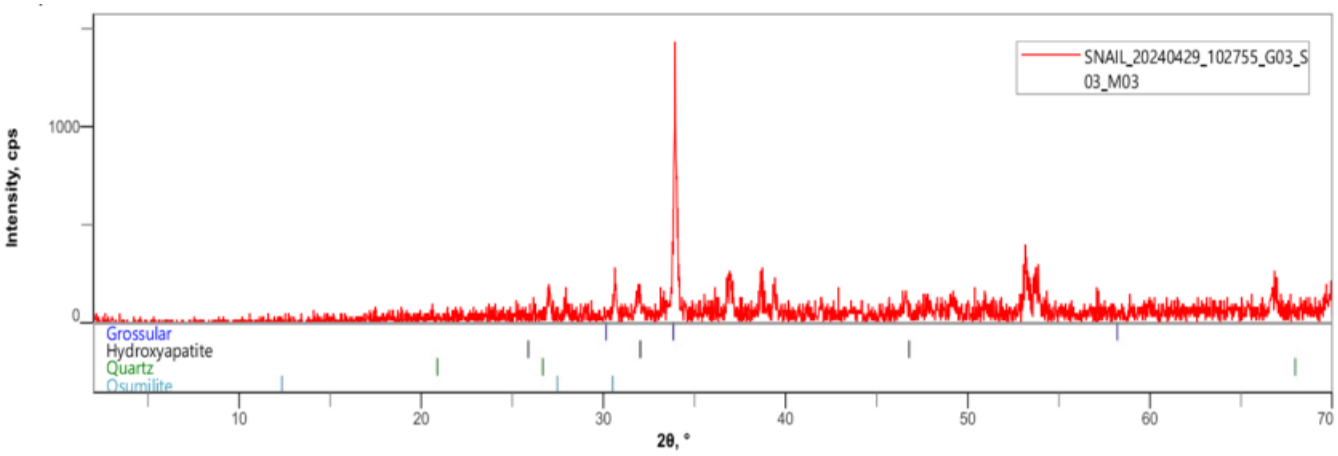

Figure 7b: XRD pattern illustrating the crystalline structure of snail-derived chitosan

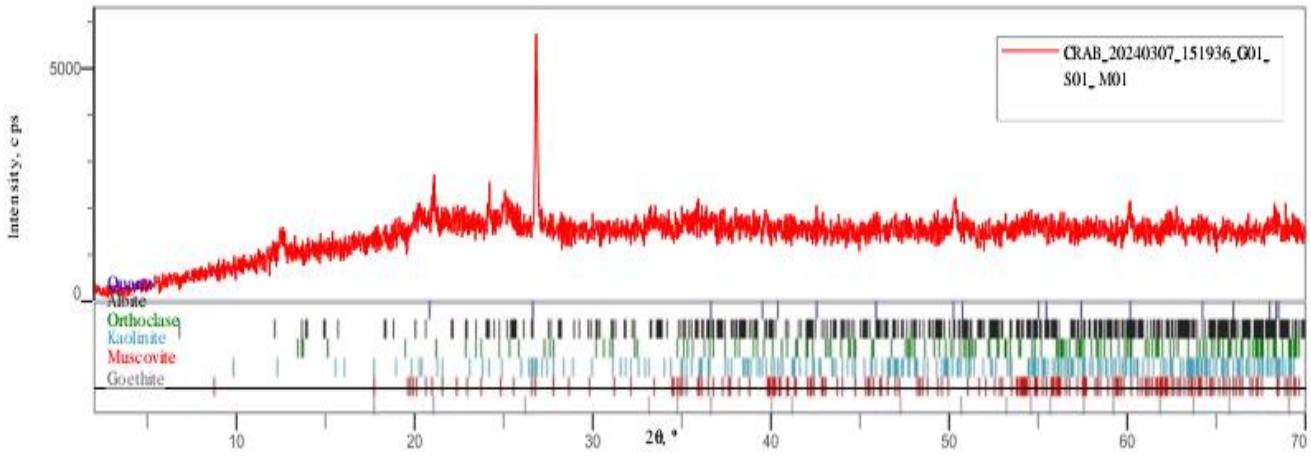

Figure 7c: XRD pattern illustrating the crystalline structure of crab-derived chitosan

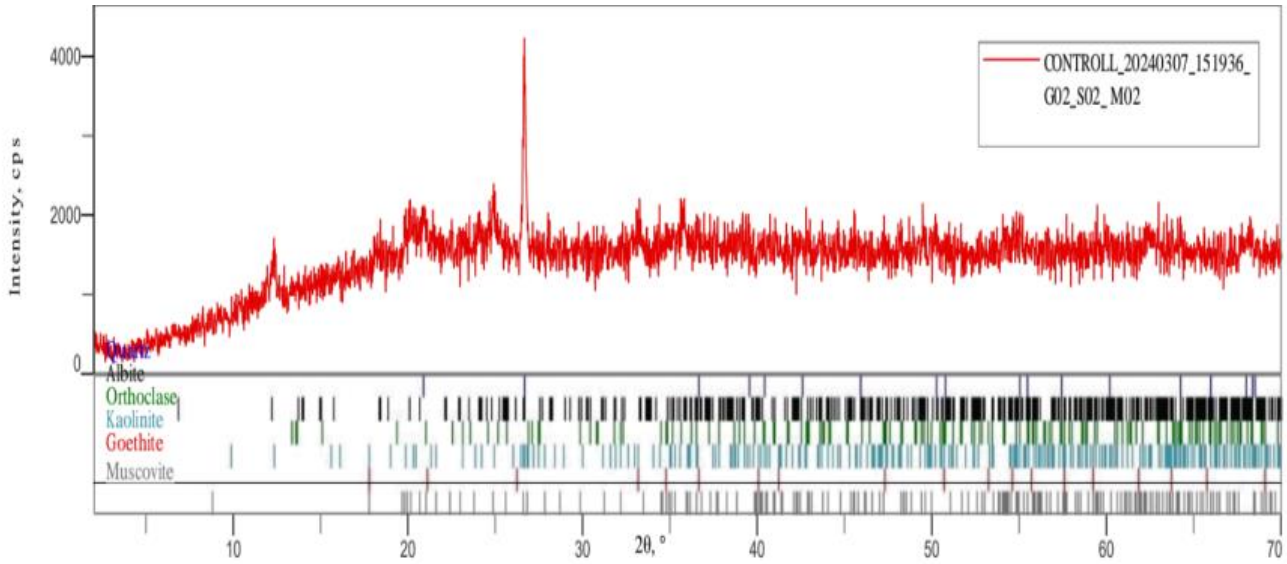

Figure 7d: XRD pattern illustrating the crystalline structure of the commercial chitosan

Ash content and moisture content

Chitosan's ash content is a good measure of its quality. It is also a measure of the efficiency of the demineralization process. A reduced ash content signifies higher quality and refinement of chitosan. Chitosan from snail shells was seen to have a value of 0.51%, as seen in Table 5. This could be compared to results from Adekanmi *et al.*,[25] (0.14 – 0.75%). This reveals an effective demineralization process. It is also important for biomedical uses where impurities could provoke inflammatory responses.[61] The ash content of chitosan from crab shells is 0.47%. It could be compared to the results (0.6%) obtained from Hao *et al*[62]. Chitosan from periwinkle shells was observed to have a significantly high ash content ($p < 0.05$). This could be due to the high level of impurities, incomplete demineralization, or shell composition differences. This result is similar to the one obtained by Oyawoye *et al.*,[63]

Table 5: Ash content and moisture content of the commercial and derived chitosan samples

Samples	Ash content (%)	Moisture content (%)
Commercial chitosan	0.42 ± 0.04	1.293 ± 0.06
Snail chitosan	0.51± 0.01	1.950 ± 0.04
Crab chitosan	0.47 ± 0.02	1.682 ± 0.02
Periwinkle chitosan	10.46 ± 0.01	5.891 ± 0.04

Chitosan's moisture content is a very important parameter as it depicts its hygroscopic nature, i.e., its ability to absorb moisture from the environment.[64] Its moisture content also depicts its propensity to microbial growth. A high moisture content makes the chitosan more prone to microbial growth.[65] Also, biomedical applications such as wound healing would require that the chitosan has sufficient moisture content for better healing of wounds.[66] Chitosan derived from snail shells was observed to have an average moisture content of 1.95. These results can be compared to results by Egbeneje *et al.*,[50] This low moisture content depicts a high-quality chitosan with a high extent of deacetylation. The commercial chitosan, as well as chitosan from shells of crabs, were also observed to have low moisture content, 1.293 and 1.682. These results may be compared to results by Ahing and Wid.[67] The difference may be due to the drying of the chitosan after its formulation. The results obtained from crabs may be compared to those of Metin *et al.*[68] The moisture content supports better stability during storage. A slight increase was observed in the moisture content of periwinkle shells (5.891%). This result could be compared to that of Agoha (2007). This higher moisture content could be attributed to its porous surface morphology and residual water drying conditions.[69]

Solubility and viscosity

The solubilities and viscosities of the extracted samples and the commercial chitosan are summarized in Table 6. The commercial chitosan showed significantly higher solubility ($p < 0.05$) than the locally extracted samples. This could be attributed to a higher degree of purity and optimized processing conditions. The solubility of chitosan extracted from shells of snails, periwinkles, and crabs is 72%, 86%, and 70%, respectively. These results could be compared to those of Fernandez-Kim,[70] Tamzi *et al*,[71] and Adekanmi *et al.*[25] The variation in the solubility of the various chitosan from seafood shells could be attributed to the varying degrees of deacetylation, levels of impurities, and different shell compositions. The lower solubility of snail-derived chitosan could be attributed to its lower DDA and potential structural impurities, such as residual minerals or proteins, which can hinder complete dissolution.

Table 6: Solubility and viscosity of the commercial and derived chitosan samples

Sample	Solubilities (%)	Viscosities (mPa.s)
Commercial chitosan	100 ± 0.01	481.2 ± 0.04
Snail chitosan	72 ± 0.02	258.2 ± 0.06
Crab chitosan	86 ± 0.02	219.6 ± 0.02
Periwinkle chitosan	70 ± 0.01	60.21 ± 0.04

The commercial chitosan was observed to have a significantly high viscosity (481.2 mPa.s). This high viscosity correlates with its high molecular weight and degree of deacetylation, which enhances intermolecular hydrogen bonding and polymer chain entanglement. These factors are advantageous for controlled drug release and scaffold formation in tissue engineering. Conversely, the lower viscosity of periwinkle-derived chitosan could make it considerable for applications like sprayable formulations or fast-dissolving wound dressings, pending functional testing.

Dissolution of chitosan in solvents

The dissolution of chitosan in some selected solvents is revealed in Table 7. The extracted and commercial chitosan samples exhibited similar dissolution profiles. From Table 7, it is observed that the

chitosan samples are insoluble in water. This is because chitosan is known to have a poly-cationic nature. This enhances its insolubility in neutral water as the amino groups form strong intramolecular bonds. Chitosan is insoluble in water due to the absence of protonation of its amine groups at neutral pH.[72] All chitosan samples were observed to be insoluble in methanol, ethylacetate, and ethanol. This is due to the non-acidic nature of the solvents. Chitosan requires protonation to interact properly in a solvent, and this is possible in acidic conditions.[73] Chitosan from all sources was also observed to be insoluble in chloroform. This is due to the polar nature of chitosan, which makes it incompatible with non-polar solvents like chloroform. Additionally, strong intramolecular hydrogen bonding in chitosan further reduces its solubility in such solvents. The strong hydrogen bonds between chitosan are also a contributory factor to the insolubility of chitosan in chloroform. A summary of the different samples of chitosan, some of their characteristics, and their suggested applications in pharmaceutics and pharmaceutical technology, based on these characteristics, is shown in Table 8. However, it should be noted that while physicochemical characterization provides insight into potential applications, functional studies such as antimicrobial testing, cytotoxicity assays, and drug loading efficiency evaluations are needed to fully validate these hypotheses. Future research will focus on evaluating these biological properties to confirm the therapeutic potential of the chitosan samples extracted from these seafood sources.

Table 7: Dissolution of the commercial chitosan and derived chitosan samples in some solvents

Chitosan sample	Chloroform	Ethylacetate	Water	Ethanol	Methanol
Snail	Insoluble	Insoluble	Insoluble	Insoluble	Insoluble
Commercial	Insoluble	Insoluble	Insoluble	Insoluble	Insoluble
Crab	Insoluble	Insoluble	Insoluble	Insoluble	Insoluble
Periwinkle	Insoluble	Insoluble	Insoluble	Insoluble	Insoluble

Table 8: Summary of physicochemical characteristics and suggested pharmaceutical applications of commercial and extracted chitosan samples

Characteristic	Commercial chitosan	Chitosan from snail	Chitosan from crab	Chitosan from periwinkles
Degree of Deacetylation	75% enhances solubility and mucoadhesion, making it useful in various drug delivery systems	62.5% Good solubility	71% Good solubility and biocompatibility	86.6% Improved solubility and bioactivity
Particle morphology	Small particle size, high homogeneity, ideal for nanoformulations, drug encapsulations, and uniformity in dosage forms	Irregular	Smooth, homogenous surface with moderate porosity suitable for drug encapsulation and controlled release	Irregular, porous, and rough.
Thermal Stability	Low suitable for applications where quick degradation or dissolution is not a requirement	High Ideal for sustained and controlled-release formulations	Low best suited for formulations that do not require high temperature and processing	Low, ideal for formulations that do not require high heat stability and for topical applications, oral fast-dissolving formulations
Crystallinity	High ideal for controlled or sustained release formulations due to a slow dissolution rate.	Low-suitable for controlled drug release over time	Moderate, well-ordered crystalline structures- Ideal for gradual drug release and targeted delivery	Moderate Suitable for moderate-release profiles, controlled drug delivery systems, and coatings
Moisture content	Low indicates it is stable and resistant to water absorption, good for dry formulations like tablets or powders	1.95% can be used in formulations requiring controlled moisture, e.g., wound healing, gels	Low moisture content, suitable for dry formulations and stable storage	5.891% Its comparatively higher moisture content might suggest that it could be used in hydration-sensitive

				applications, like hydrogels or wound dressings, provided that further formulation studies are conducted.
Viscosity	High suitable for gel formulations, thick suspension, or coating applications	Medium Useful for gel formulations or applications requiring moderate flow.	Medium Suitable for gel formation or thicker formulations	Low Good for liquid formulations, suspensions, or liquid gels

Conclusion

This study successfully extracted and characterized chitosan from the shells of three seafood sources; crabs, snails, and periwinkles collected from the Uyo metropolis, Nigeria, and compared them with a commercial chitosan sample. Significant variations in physicochemical properties, including the extent of deacetylation, solubility, viscosity, thermal stability, crystallinity, and surface morphology, were observed among the samples, underscoring the influence of biological source and extraction method on chitosan quality. Periwinkle-derived chitosan exhibited the highest degree of deacetylation and solubility, suggesting potential for applications requiring high water compatibility, such as wound healing formulations and fast-dissolving drug carriers. Snail-derived chitosan demonstrated superior thermal stability and low crystallinity, characteristics desirable for biodegradable implants or controlled-release systems. Crab-derived chitosan displayed moderate crystallinity and high purity, making it suitable for structural biomedical applications. The availability of bioactive compounds identified by GC-MS analysis further suggests that these locally sourced chitosan samples may offer inherent antimicrobial or therapeutic properties, enhancing their potential value in pharmaceutical and biomedical industries. However, while physicochemical characterization provides a foundation, functional validation through antimicrobial, cytotoxicity, and biocompatibility studies remains essential. Future research will focus on evaluating these biological properties to fully establish the application-specific potentials of chitosan derived from Nigerian marine resources. These findings not only demonstrate the feasibility of producing quality chitosan from indigenous seafood waste but also highlight an opportunity to develop sustainable biomaterials locally, reducing reliance on imported commercial products.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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