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EXTRACTION AND CHARACTERIZATION OF CHITOSAN FROM GIANT AFRICAN LAND SNAIL'S SHELLS

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ABSTRACT: Chitosan, a biopolymer derived from chitin, has garnered significant attention due to its unique properties, eco-friendly nature, and diverse applications in various industries owing to its remarkable properties. This study focuses on the extraction and characterization of chitosan from giant African snail shells. Various experiments were designed to optimize the extraction process, resulting in a chitosan yield of 61.37%, a moisture content of 2.02%, and a degree of deacetylation (DD) of 68.24%. The physicochemical properties of the extracted chitosan nanoparticles were characterized, revealing important parameters for its potential applications. Fourier Transform Infrared Spectroscopy (FTIRS) analysis indicated the presence of functional groups such as O-H, N-H, C≡C, C=C, and N-H bending, corroborating previous studies. Scanning Electron Microscopy (SEM) revealed the chitosan particles' polygonal shape and rough surface, suggesting their suitability for adsorption applications and heavy metal trapping. X-ray Diffraction (XRD) analysis demonstrated a semi-crystalline nature of the chitosan, with a sharp peak at 34° (2θ) and other suppressed peaks. These findings confirm the successful synthesis of chitosan from giant African snail shells and highlight its potential for various applications, supported by its favorable physicochemical properties.

INTRODUCTION: Global research has increasingly focused on environmental pollution issues, driven by concerns over its adverse effects on both animals and humans. Industrial waste, in particular, has emerged as a significant contributor to environmental degradation, posing risks to human health and wildlife. Among the diverse sources of waste, snail shells have drawn attention due to their abundance and the challenges they pose to the environment [14-23].

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The giant African land snail, belonging to the family Achatinidae, is among the largest terrestrial gastropods, boasting an average lifespan of 5-7 years, though some individuals have been documented to live up to 10 years [14-23].

Achatina fulica, a notable species of this genus, is renowned for its invasiveness, capable of consuming over 500 plant species. Despite being listed among the 100 most harmful invasive alien species globally, *A. fulica* continues to thrive, adapting easily to diverse environments beyond its natural habitat [14-23]. While the inner content of snails holds nutritional value for humans, their shells contribute to environmental nuisance after consumption. Notably, snail shells offer a promising source for chitosan production, a

valuable material with various industrial applications. The southern region of Nigeria harbors diverse snail species, with *Archachatina fulica* being a common delicacy [14-23].

FIG. 1:

The shells of these snails, rich in carbohydrates, offer a higher yield of chitosan compared to seafood waste. However, despite their potential, there's limited research on utilizing snail shells for chitosan production. Moreover, the rainy season exacerbates environmental pollution as snails proliferate, leading to indiscriminate shell disposal mainly by establishments like restaurants and hotels Crustacean shells also contribute significantly to waste production, with approximately 8.4 million tonnes generated in 2017. The accumulation of these shells poses a severe environmental threat, necessitating recycling efforts. Chitosan production *via* chemical deacetylation of chitin presents a viable solution to mitigate environmental pollution while harnessing economic opportunities [1, 4, 10, 16, 23].

Chitin, a structural component found in arthropods, mushrooms, algae, coral, and nematodes, offers diverse applications across various industries. Its degree of acetylation is crucial for determining its physical properties and functional characteristics. Notably, chitosan derived from shells of crustaceans like crabs, shrimps, and prawns serves as an organic polysaccharide with multifaceted utility, ranging from medicine to biotechnology.

The exploration of waste materials like snails and crustacean shells for chitosan production represents a sustainable approach to environmental management and resource utilization, offering economic benefits alongside environmental protection materials [8, 19, 25, 26]. Chitosan, a linear polysaccharide composed of randomly distributed β-(1→4)-linked D-glucosamine and N-acetyl-D-glucosamine (acetylated unit), is derived from the chitin shells of crustaceans like shrimp, through treatment with an alkaline substance such as sodium hydroxide [10, 11]. Despite its solubility challenges, higher molecular weight chitosan with increased degree of deacetylation (DD) forms stable complexes with DNA/RNA molecules, enhancing delivery efficiency [10].

Commercial production of chitosan involves the deacetylation of chitin, primarily sourced from crustacean exoskeletons and fungal cell walls. Fourier Transform Infrared Spectroscopy (FTIR) can determine the degree of deacetylation (DD), which typically ranges from 60 to 100% in commercial chitosans, with an average molecular weight of 3800–20,000 daltons [20].

The amino group in chitosan exhibits significant protonation in neutral solutions due to its pKa value (~6.5), enhancing solubility and bioadhesive properties, particularly on negatively charged surfaces like mucosal membranes. The free amine groups on chitosan chains facilitate the formation of crosslinked polymeric networks with dicarboxylic acids, improving mechanical properties [5, 17].

Chitosan, derived from chitin, exhibits varied physicochemical properties crucial for its diverse applications. These properties include fat binding capacity (FBC), water binding capacity (WBC), solubility, molecular weight, ash content, moisture content, and degree of deacetylation. Unlike chitin, chitosan's solubility in organic acids like acetic acid is a notable distinction, attributed to its higher content of protonated amino groups. The degree of deacetylation, achieved through the removal of acetyl groups from chitin, is pivotal as it influences chitosan's physicochemical properties, biodegradability, and applications.

Chitosan's molecular weight varies with raw material sources and processing methods, affecting its stability and degradation under conditions like high temperature and shear stress [5]. In agricultural and horticultural contexts, chitosan serves as a plant growth enhancer and biopesticide, eliciting innate defense responses in plants against pathogens and pests while promoting growth and

nutrient uptake. Its use as a seed treatment enhances immunity in developing roots, combating parasitic nematodes without harming beneficial organisms [13, 18, 21 28, 30]. Chitosan also finds application in water filtration, where it binds sediment particles and removes heavy minerals, dyes, and oils, offering an eco-friendly solution for water purification [24, 32].

Moreover, chitosan serves various industrial purposes, including winemaking, wound dressing, and medical device development. It acts as a fining agent in winemaking, reducing polyphenolic oxidation and controlling spoilage yeast [9]. In medical settings, chitosan-based products exhibit hemostatic properties, promote wound healing, and serve as drug delivery systems and tissue engineering materials [6, 27, 31].

Additionally, chitosan and its derivatives hold promise in nanotechnology, offering potential applications in nanofiber membranes, metallic nanomaterials for wound healing, and adjuvants for vaccine delivery systems [22, 29].

MATERIALS AND METHODS:

Sample Collection and Preparation: Snail shell samples were collected at the wurukum market in Makurdi metropolis, Benue State, Nigeria. The sample was properly washed using tap water, sundried, and subsequently oven-dried at 100^{o}C for 1 hour. The sample was further pulverized using a wooden mortar and pestle, sieved with a 2mm sieve, and stored in an air-tight plastic container for further analysis.

Extraction of Chitosan: 50 g of snail shell powder was weighed with an analytical balance. Then, deproteinization was carried out using 200 mL of 10% NaOH with stirring and heating at 100°C. The treatment was carried out for a duration of 5 hours with the use of a rotating mechanical stirrer. The treated sample was washed with distilled water and filtered until the sample was at a neutral pH.

Demineralization was carried out on the neutralized sample using 250 mL of 1 M HCl solution with stirring and heating at 100°C for 30 minutes using a rotating mechanical stirrer. The sample was again washed with distilled water and filtered until it had a neutral pH. The product from demineralization underwent deacetylation with the use of 100 mL of 50% NaOH with stirring and heating at 100°C for 6 hours using a rotating mechanical stirrer. The sample was filtered and washed with distilled water until it had a neutral pH. It was then dried at room temperature for 48 hours, collected, and stored in sample bottles [15].

Physicochemical Analysis:

Estimation of Percentage Yield: The percentage yield of chitosan was determined using the ratio of the masses of the chitosan and crude material in the formula [1, 2].

% Yield = (Practical yield) / (Theoretical yield) × 100

Moisture Content of the Chitosan: An empty crucible was weighed (dried). 1 g of the sample was weighed into the clean dry empty crucible, and the weight of the crucible and the sample was recorded. The crucible and the chitosan sample were placed in an oven for 30 minutes at 100°C. After drying, the sample was allowed to cool in a desiccator for 30 minutes and reweighed [7]. The moisture content was calculated using the formula provided by Agarwal *et al.* (2018) [1, 2].

Top of Form:

Moisture Content (%) = (wet weight-dry weight) / (wet weight) × 100

Degree of Deacetylation (DD): The degree of deacetylation (DD) of the chitin samples was calculated using the method proposed by Domszy and Roberts (1985) presented in the equation below:

$DD = 100-((A_{1655}/A_{3450}) \times 100) / 1.33$

A1655 represented amide absorbance at 1655 cm^{-1} and A3450 represented hydroxyl absorbance at 3450 cm-1 while 1.33 denoted the value of the ratio of A_{1655}/A_{3450} [2].

Characterization:

Fourier Transform Infrared Spectroscopy (FTIR): FTIR analysis was conducted using Agilent Technologies Cary 630 FTIR. Infrared rays of various wavelengths were directed onto the sample, leading to absorption of radiation by the sample and transmission of the remaining radiation. This process generated a spectrum depicting the absorption and transmission of the sample

molecule, corresponding to the frequencies of vibrations between the atoms' bonds constituting the sample material. The spectrum obtained from FTIR analysis was utilized to identify the functional groups present in the sample [8, 25, 26].

Scanning Electron Microscopy (SEM): The scanning electron microscopy (SEM) was performed to examine the physical structure change of samples using SEM model Phenom ProX, by phenom World Einhoven, the Netherlands. Sample was placed on double adhesive which was on a sample stub, was coated sputter coater by quorum technologies model Q150R, with 5nm of gold.

Thereafter it was taken to the chamber of SEM machine where it was viewed *via* NaVCaM for focusing and little adjustment, it was then transferred to SEM mode, was focused and brightness contrasting was automatically adjusted, afterward the morphologies of different magnification was store in a USB stick [8, 25, 26].

X-ray Diffraction (XRD): The samples were registered in a zero-background sample holder to avoid external background interferences. The diffractograms were registered in the range of 7–90 (2h) in a step scan mode of 0.026261 at a counting time of 17.34 s per step [8, 25, 26].

RESULTS AND DISCUSSION: In this study, the experiments were designed to aid the extraction of chitosan from land snail shells. The results on physicochemical properties and characterization techniques of the prepared chitosan are given in the tables and figures below;

TABLE 1: PHYSICOCHEMICAL CHARACTERIZATION OF NANOPARTICLES

Physicochemical Parameter	Obtained Value
Percentage yield (%)	61.37
Moisture Content (%)	2.02
Degree of deacetylation (DD) (%)	68.24

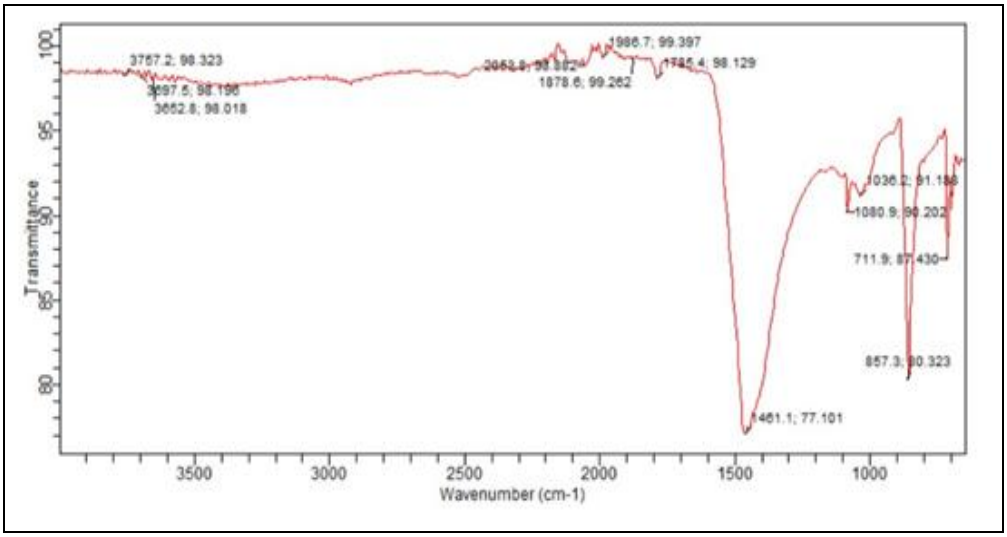

FIG. 2: FTIR-SPECTRA OF CHITOSAN

TABLE 2: FTIR VALUES OF CHITOSAN

S. no.	Wavelength(cm^{-1})	Functional Group
1	3757.2	O-H stretch
2	3652.8	O-H and N-H Stretch
3	2052.8	C≡C Stretch
4	1986.7	C=C Stretch
5	1785.4	N-H Bend
6	1461.1	C-H Bend
7	1080.9	C-O Stretch
8	1036.2	C-N stretch
9	857.3	N-H out of plane bend
10	711.9	C-O out of plane bend

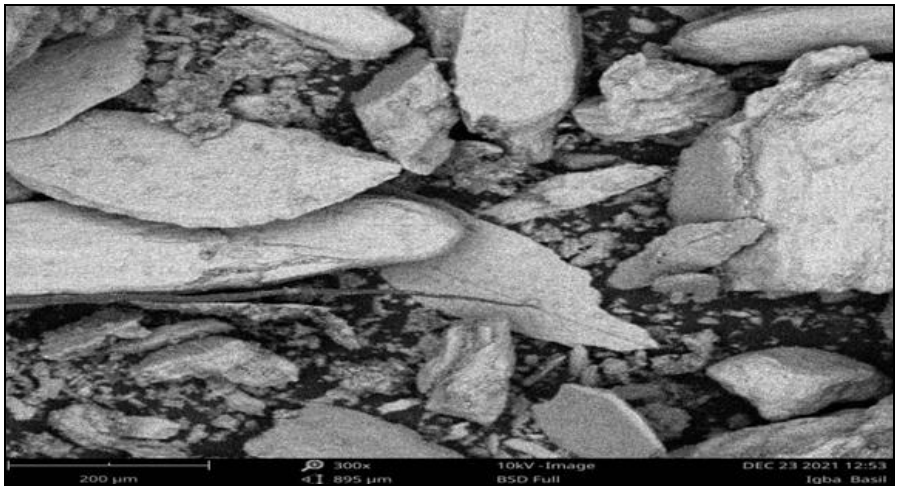

FIG. 3: SEM SPECTRUM OF CHITOSAN (×300)

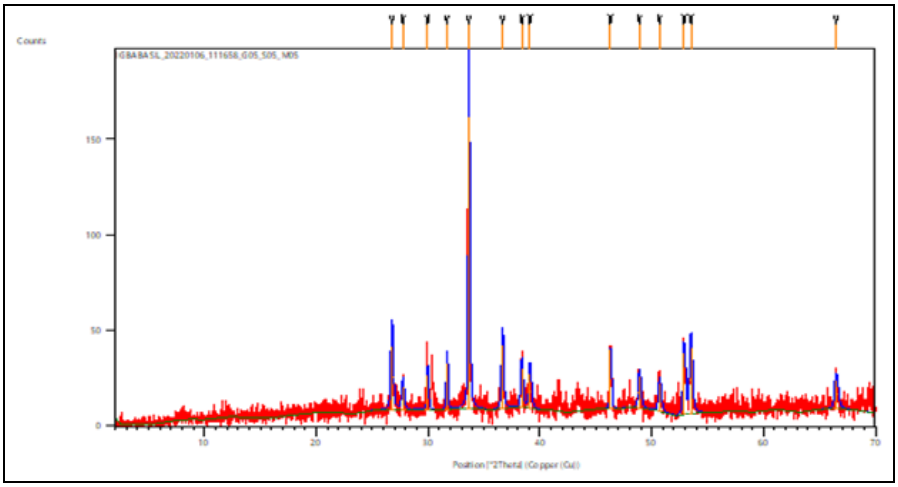

FIG. 4: XRD-SPECTRA OF CHITOSAN

DISCUSSIONS: The physicochemical characterization of the chitosan provides essential insights into their properties and potential applications. In this study, the prepared chitosan exhibited a moderate percentage yield of 61.37%, indicating that the preparation process is fairly efficient but may benefit from further optimization. The low moisture content of 2.02% suggests that the chitosan is stable and suitable for long-term storage, which is crucial for maintaining their quality and preventing degradation. Additionally, the degree of deacetylation (DD) at 68.24% shows that the chitosan have a good balance of solubility and bioactivity, making them suitable for applications such as drug delivery and antimicrobial agents. The functional groups, morphology and crystalline structure of chitosan were determined using Fourier transform infrared spectroscopy (FTIRS), Scanning electron microscopy (SEM) and X-ray diffraction (XRD) respectively.

The FTIR spectrum of chitosan indicated various functional groups at different peaks. The bands observed at 3757.2 cm^{-1}, 3652.8 cm^{-1}, 2052.8 cm^{-1}, 1986.7 cm^{-1} and 1785.4 cm^{-1} can be attributed to O-H stretch of alcohol groups; NH stretch of amide group and OH stretch of alcoholic group; C≡C stretch of alkyne groups, C=C stretch of the aromatic group and the chitosan characteristic N-H bending of amine groups. The bands present in the fingerprint region at 1461.1 cm^{-1}, 1080.9 cm^{-1}, 1036.2 cm^{-1}, 857.3 cm^{-1} and 711.9 cm^{-1} can be assigned to C-H bend of alkanes; C-O stretch of alcohol; C-N stretch of aliphatic amines; N-H out of plane bend and C-O out of plane bend respectively. This result can be favorably compared with that of Oyekunle and Omoleye (2019) and Bamiro *et al.* (2021) [7, 8] who obtained the above values at 3718.88 cm^{-1}, 3637.00 cm^{-1}, 2109.00 cm^{-1}, 1982.00 cm^{-1}, 1419.66 cm^{-1}, 1080.17 cm^{-1} and 1028 cm^{-1} respectively.

The scanning electron micrograph showed that the chitosan particles produced are polygonal in shape with a rough surface. This non-crystalline nature makes them effective for adsorption applications or provides a favorable environment for trapping heavy metals. This result can be related to that of Bamiro *et al.* (2021) [7] who had polygonal and rough surfaces. The XRD of chitosan shows a sharp peak at 34° (2θ) and other suppressed peaks which shows that the chitosan produced is semi-crystalline. The value of this intense peak 34° (2θ) corresponds with that of Bamiro *et al.* (2021) [7] who had theirs at 33.2° (2θ).

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