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COMPARATIVE STUDIES OF THE PHYSICOCHEMICAL PROPERTIES OF CHITOSAN FROM COMMERCIAL AND LOCALLY SOURCED PERIWINKLE SHELL FROM AKWA-IBOM

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ABSTRACT

*Chitosan is a versatile bio-polymer of D-glucosamine and N-acetyl-D-glucosamine derived from the deacetylation of chitin, with relevance in several applications such as environmental, textile, food, biomedical, and pharmaceutical industries. Chitosan extracted from periwinkle shell (CPS), a biowaste via deproteination, demineralization and deacetylation, was characterized alongside commercial chitosan (CC) purchased from MarkNature, USA. The aim was to utilize local waste, periwinkle shell, as an alternative source of chitosan and compare its physicochemical properties with those of commercially procured chitosan. Characterization was done using Fourier Transform Infrared Spectroscopy (FTIR). Moisture, ash and protein contents were determined using standard methods. The percentage yield, solubility, degree of de-acetylation, and water and fat binding capacities were also determined using their standard methods. The FTIR spectra of chitosan for CC and CPS gave characteristic free hydroxyl (OH) bands at 4037.00 and 4047cm^{-1} respectively, aliphatic –OH- absorption bands of 3430.17 and 3458 cm^{-1}, aliphatic CH_3 absorption bands of 2966 and 2927 cm^{-1}; NH absorption (bending) bands of 1309 and 1320.60 cm^{-1} and a -CO/NH_2 bending bands of 1686.39 and 1643.30 cm^{-1} respectively. CC and CPS had moisture content of 6.94 ± 0.05 and 8.65± 0.08 %, ash content of 1.87 ±0.03 and 1.61±0.01 % and protein content of 1.70**±0.08** and 2.80**±0.012** %. The percentage yield of CPS was 58**± 0.18** % and was soluble in 1% acetic acid with a degree of de-acetylation of 87.81 **±0.09**%. The water binding capacities of the CC and CPS were 970 ±0.11 and 244± 0.071 %, while their fat binding capacity were 177.61±0.012 and 213.7±0.014 %, respectively. The study has shown that chitosan prepared from periwinkle shell showed good physicochemical properties when compared favourably with commercial chitosan.*

KEYWORDS: Characterization, Chitosan, Periwinkle shell, Physico-chemical properties

INTRODUCTION

Food processing generates a large quantity of by-products (waste), which can cause environmental and human problems. Most are still underutilized and are substances of high practical value (Hamed *et al.*, 2016). The seafood industry generates about 106 tons of waste annually, most

destined for composting or converted into low-value-added products such as animal feed and fertilizers (Schmitz et al., 2019). The crustacean shell is one of the prominent sources of chitin and chitosan because of the abundance of the biomaterial and the large-scale industrial extraction process (Adekanmi *et al.,* 2023). According to the Food and Agriculture Organization of the United Nations, 2019 report, the waste generated in 2017 from crustacean shells was 8.4 million tonnes (Adekanmi *et al.,* 2023). As a result of large amounts of shells littering the environment has led to the urgent need for recycling, which results in the production of chitosan through chemical deacetylation of chitin (Amoo *et al.,* 2019).

Approximately 2000 tons of chitosan is produced annually, whose primary extraction source is shrimp and crab shell residues (Muñoz *et al.,* 2018). The shell of selected crustaceans was reported by Abdulwadud *et al*. to consist of 30%–40% protein, 30%–50% carbonate and phosphate of calcium and 20%–30% chitin (Kumari *et al.,* 2015). Like other crustaceans and molluscs, Periwinkle shell wastes contain chitin, which can be further deacetylated into chitosan (Gbenebor *et al*., 2017). One typical shell food consumed in southern Nigeria is Periwinkle (*Tympanotonus fuscatus*) as a source of protein (Ekop *et al.,* 2021). They are gastropods of the phylum Mollusca. They are found in Nigeria's lagoons, estuaries, mangroves, and swamps (Paul *et al.,* 2022). The empty shells are poorly utilized, adding to the environmental menace (Ugoeze & Chukwu, 2015). Periwinkle shells are abundant in Nigeria and can be used as a chitosan source.

Chitosan is defined as a linear polysaccharide formed by the random distribution of two monosaccharides, D-glucosamine and D-N-acetyl-glucosamine, which are joined together by a ß-(1–4) bond (Jayakumar *et al*., 2010). Chitosan is a natural polycationic polysaccharide obtained from the deacetylation of chitin from crustaceans, molluscs and some fungi. Chitosan is chemically and thermally difficult to degrade but is highly stable (Amoo *et al*., 2019). In its crystalline form, chitosan is usually insoluble in aqueous solutions above pH of 7; however, in dilute acids (pH6.0), the protonated free amino groups on glucosamine facilitate solubility of the molecule (Di Martino *et al.*, 2005). Chitosan's charge density depends on the degree of acetylation and pH of the media (Aranaz *et al*., 2021). Chitosan has some advantageous properties, such as biocompatibility, biodegradable polymer of high molecular weight, nontoxic, and antimicrobial activity, that encourage its applications in many fields such as agriculture, paper industry, food and textile industries, pharmaceutics, biochemistry, biotechnology, cosmetics, biomedical applications, environment, and water

treatment (Das *et al.,* 2024). This study aimed to utilize local waste, periwinkle shell, as an alternative source of chitosan and compare its physicochemical properties with those of commercially procured chitosan.

MATERIALS AND METHODS

Sample collection and preparation: The fresh periwinkle shells were collected from the Esuk Itsu river bank in Urua ato, Ikot Ekpene of Akwa Ibom state, Nigeria. The periwinkle samples were washed and cleaned to remove dirt and other extraneous materials. They were sun-dried for 120 hours, after which they were ground to a fine powder and stored in clean, dry plastic bags at an ambient room temperature of 25°C.

Method of Analysis

Preparation of Chitosan

Chitosan preparation involves three steps: demineralization, deproteinization, and deacetylation, as prescribed by Anand *et al.,* (2015).

Demineralization: One hundred grams of the ground periwinkle shell was soaked in 4 % HCl at room temperature in the solid-to-solvent ratio of 14:1 (w/v) for 24 hours. It was washed in the acid solution until no bubbles were seen, and no colour change was observed. The shell was washed with deionized water until a relatively neutral pH was obtained and dried to constant weight.

Deproteinization: The demineralized powder was treated with 5% NaOH at 60 °C for 24 hours with a solid-to-solvent ratio of 12:1 (v/w). After incubation, the slurry was washed to a neutral pH using deionized water and oven-dried at 80°C to obtain chitin.

Deacetylation: The chitin was deacetylated with a 70 % NaOH solution with a solid-to-solvent ratio of 1:14 (w/v) at 65 °C for 72 hours. The residue was washed with deionized water to neutrality to obtain chitosan. The chitosan was then filtered, oven-dried for 4 hours at 70°C and finely ground for characterization.

Proximate Profile of the Chitosan: The proximate composition (moisture, ash, protein, and fat) of chitosan was analyzed according to the AOAC (2015) method with some modifications.

Properties analysis of the chitosan

Determination of yield: The yield was determined by taking the dry weight of the periwinkle shell before treatment and the dry weight of the chitosan. The yield of chitosan was determined according to the formula**:**

$$\boldsymbol{chitosan\ yield} = \frac{\boldsymbol{weight\ of\ chitosan\ (g)}}{\boldsymbol{weight\ of\ periwinkle\ (g)}} \boldsymbol{X\ 100}$$

Degree of Deacetylation (DD): This was conducted using a modified titration method described by [Kjartansson, 2008]. A sample of chitosan (0.1 g) was dissolved in HCl (0.06M) of 25ml at room temperature for 1 hour. Titration was done using 0.1M NaOH while stirring to obtain a pH of 3.75 after initial solution dilution to 50ml. At 3.75 pH, the NaOH volume required was recorded as V_1. Titration was continued to pH 8, and the total volume of NaOH (0.1 M) was recorded as V_2. The degree of deacetylation (DD) was calculated using

$$D.D = \frac{161.16(V2-V1)M}{W1}$$

Here, 161.16 represents the mass of the chitosan monomer, V1 is the volume of NaOH at 3.75 pH, V2 is the volume of NaOH at pH 8, M is the molarity of NaOH, and W1 is the sample mass immediately after moisture is removed.

Solubility determination: The solubility of the chitosan was determined by adding 200 mg of the chitosan in 200 ml of distilled water. The process was repeated with 200 ml of 1 % acetic acid solution. The solubility of the chitosan was observed in the two solutions.

Determination of water binding capacity (WBC): The Chitosan sample (0.5 g) was weighed into a centrifuge tube of 50 ml before adding 10 ml of water and mixing on a vortex mixer for 1 minute to disperse the sample. The content was left at ambient temperature for 30 minutes with intermittent shaking for 5 seconds every 10 minutes, then centrifuged at 3,200 rpm for 25 minutes. After centrifugation, the supernatant was decanted, and the tube was reweighed (Ocloo *et al.*, 2011). The water binding capacity (WBC) was calculated using the formula:

$$WBC = \frac{Water\ bound\ (g)}{Initial\ sample\ weight\ (g)}\ X\ 100$$

Fat-binding capacity (FBC): The FBC of the chitosan was determined by weighing 0.5 g of the chitosan into a 50 mL centrifuge tube, and 10 ml of gingelly oil was added, followed by mixing on a vortex mixer for 1 minute, to disperse the sample. The content was left at ambient temperature for 30 minutes with intermittent shaking for 5 seconds every 10 minutes, then centrifuged at 3,200 rpm for 25 minutes. After the centrifugation, the supernatant was decanted, and the tube was weighed (Ocloo *et al.* 2011). The fat binding capacity (FBC) was calculated using the formula.

$$FBC = \frac{Fat\ \ bound\ (g)}{Initial\ sample\ weight\ (g)}\ X\ 100$$

Fourier Transform Infrared Spectroscopy (FTIR) analysis: The prepared chitosan samples were characterized in KBr pellets using an infrared spectrophotometer model (4100 Jasco, Japan) in the 400-4,000 cm–1 range.

RESULTS AND DISCUSSION:

Table 1.0. Proximate and properties analysis of the chitosans

Sample	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Yield (%)	DD (%)	Solubility	WBC (%)	FBC (%)
CC	**6.94 ± 0.05**	**1.87 ±0.03**	**1.70 ±0.08**	**0.66 ±0.01**	**-**	***83.5**	**+++**	**970 ±0.11**	**177.61 ±0.012**
CPS	**8.65 ± 0.08**	**1.61 ±0.01**	**2.80 ±0.12**	**0.75 ±0.014**	**58 ±0.18**	**87.81 ±0.09**	**+++**	**244 ±0.071**	**213.70 ±0.014**

Note *=manufacturer's information, +++ = highly soluble, - = absent

Table 1.0 above shows the proximate composition values of moisture, ash, crude protein, crude fat content, percentage yield, solubility, degree of deacetylation, water binding capacity, and fat binding capacity of chitosan periwinkle shell waste and the commercially sourced chitosan used in this experiment.

The moisture content represents the amount of water in the sample (Tiebe *et al.*, 2018), which can influence the shelf life of a product. The moisture content of CPS (8.65 ± 0.08%) was higher than that of the chitosan CC (6.94 ± 0.05) as seen in (table 1.0). Li *et al.*, (2020) state that chitosan products should contain less than 10 % moisture.

The ash content of chitosan is a key indicator of the effectiveness of calcium carbonate removal at the demineralization stage (Nessa *et al*., 2010). In the present study, CC showed a higher ash content value (1.87 ±0.03%) than CPS (1.61 ±0.01%). Both values were higher than those reported by Kadak *et al*., (2023), whose ash content ranged between 0.98 and 1.01 %. This shows that the chitosan prepared had a standard percentage of ash content, which can be used for commercial applications.

Protein and fat content: protein content in CC and CPS were (1.70 ±0.08) and (2.80 ±0.12), respectively, which is low and shows the effectiveness of deproteinization during chitosan extraction. However, the crude content of CPS obtained aligned with that of Okafor *et al.*, (2020), whose protein content in chitosan

extracted from snail shells using sodium hydroxide as a deproteination and deacetylation agent was 2.80 %. Also, both CC and CPS had a low-fat content of 0.66 and 0.75%, which is relatively low.

The Percentage yield of the chitosan obtained from the periwinkle shell waste was 58 ±0.18% using the chemical method. The current study's result is higher than the percentage yield obtained by Sarbon *et al.*, (2015), who reported a value of 44.57 ± 3.44 % when they extracted chitosan from crab shells. This indicates that periwinkle shells are a good source of chitosan.

Degree of Deacetylation: The high degree of deacetylation depends on the high alkali concentration and heating time (Tamzi *et al.,* 2020). It determines the physical and chemical properties of the chitosan, such as encapsulation, covalent linking, and adsorption (Puvvada *et al.,* 2015). Table 1.0 shows that the DD from CPS was 87.81 ±0.09 %, which is higher than the commercial chitosan (83.5%); this indicates that the extracted chitosan has a higher purity.

Solubility is one of the most critical parameters in determining the quality of chitosan. Chitosan is an organic compound that is insoluble in water, alkaline solutions or most common organic solvents, but it is soluble to some extent in dilute aqueous acid solutions (El-hefian *et al.,* 2009). The solubility of chitosan depends on the degree of deacetylation of chitin during the chitosan extraction. The higher the degree of deacetylation, the higher the solubility of the chitosan (Wang & Zhuang, 2022) and the better quality of the chitosan is produced. The study showed that the chitosan was very soluble in 1% acetic acid, which is concordant with the findings of Okafor *et al*., (2020).

Water and Fat binding capacities: The water binding capacity is considered an essential attribute of food ingredients for formulating various value-added food products, including bakery products, to maintain their texture and structure during or after processing (Nouri *et al.,* 2016). The water binding capacity of the CC and CPS were (970 and 244) % while their fat binding capacity was (177.61 and 213.7) %) respectively. The differences in water uptake between the different chitosan products could be a result of differences in the crystallinity of the products, the number of salt-forming groups, and moisture and protein contents of the deacetylating and deproteinating materials (No *et al.,* 2000).

FTIR Characterization Results

Fourier transform infrared (FTIR) spectroscopy is a technique used to determine the vibration of functional groups in chitosan. The FTIR spectra of CC and CPS were examined, as shown in Figures 1 and 2 below. The results showed the presence of free hydroxyl groups, hydrogen-bonded O-H and C-N stretching

vibrations in amines, or C-O stretching in alcohols or esters, representing functional groups of alcohols, amines, and esters.

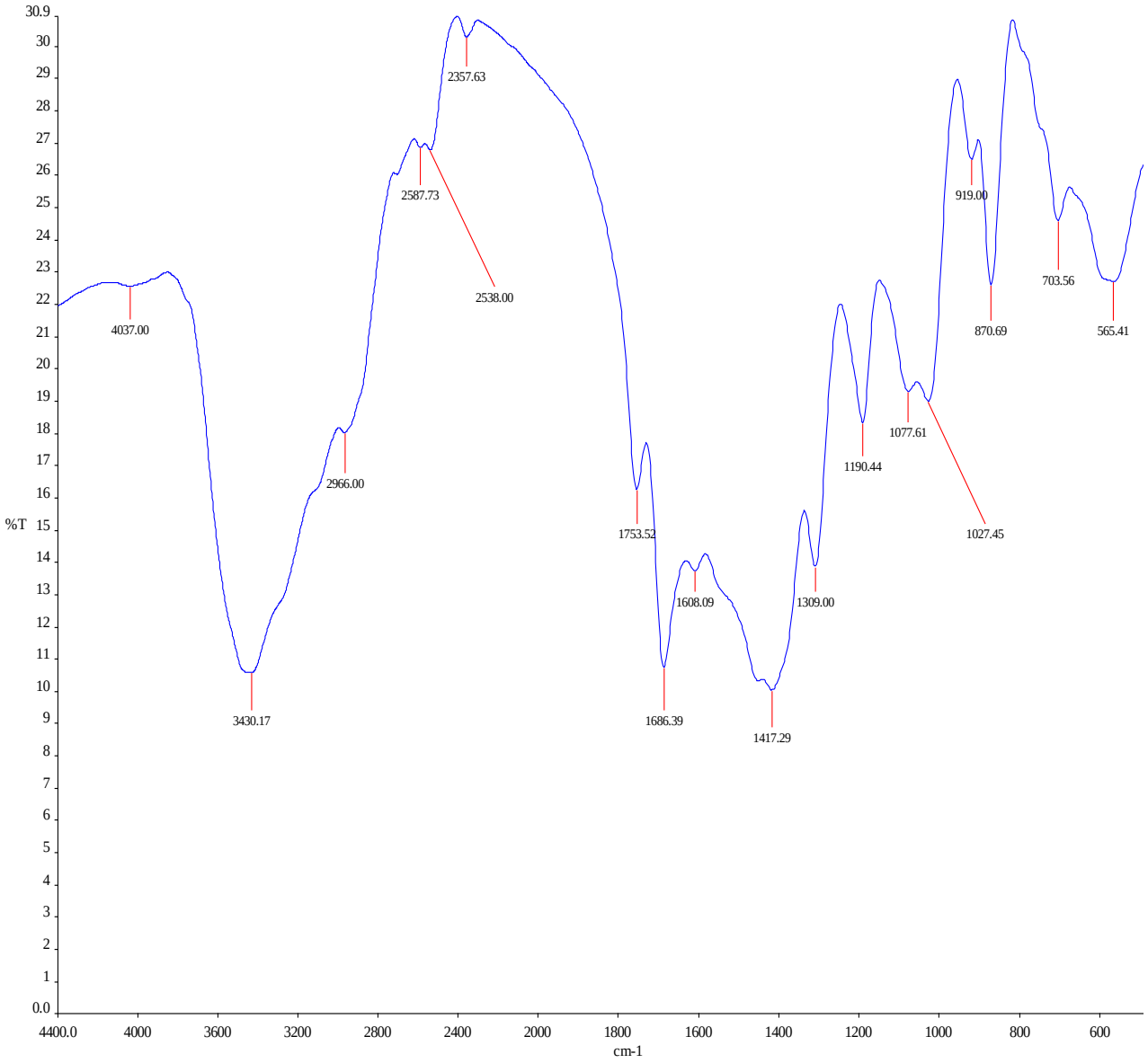

FIGURE 1: FTIR SPECTRUM FOR COMMERCIAL CHITOSAN (CC)

The IR spectrum of CC ranged from 4037-565 cm-1, as seen in (Figure 1). Figure 1 showed a strong absorption band at 3430.17 cm^{-1} due to OH and amine N-H symmetrical stretching vibrations, a peak at 2966 $cm^{-1,}$ which indicates due to symmetric -CH_2 stretching vibration attributed to pyranose ring (Pawlak & Mucha, 2003). The intensive peak around 1,686.39 cm^{-1} corresponds to C=O and the bending vibration of NH_2, which represents amide I, which is a characteristic feature of chitosan polysaccharide and also indicates the occurrence of deacetylation

(Zhang *et al.*, 2011). The peak at 1309.00 cm^{-1} was assigned to C-N stretching and NH bending vibrations present in Amide III, a component of protein (Ahyat *et al.*, 2017), while the bands at 1190.44 cm-^{1}, 1077.61 cm-^{1} and 1027.45 cm-^{1} were assigned to C–O–C group.

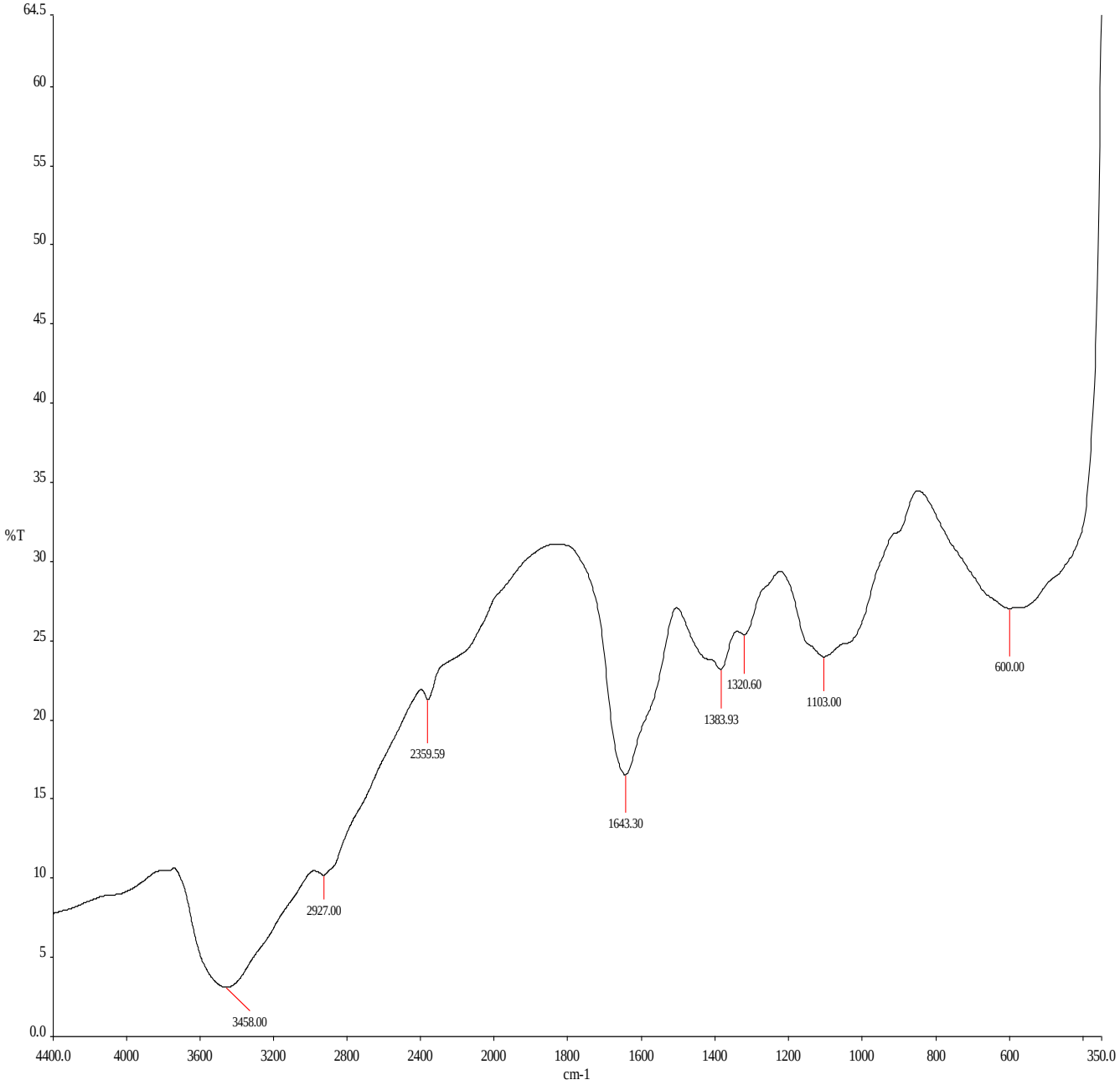

FIGURE 2: FTIR SPECTRUM FOR CHITOSAN FROM PERIWINKLE SHELL (CPS)

As shown in (Figure 2.), CPS's FTIR spectrum has a characteristic chitosan band—the spectra peaked at 3458 cm-^{1}, indicating a symmetric stretching vibration of OH and amine. The absorption peak at 2927 cm-^{1} indicates the presence of CH stretch, in line with Ahyat *et al.*, (2017) and Odili *et al.*, (2021). The

absorption band at 1643.30 cm^{-1} was due to C=O stretching (amide I) in agreement with Wanule *et al.*, (2014), while the sharp peaks at (1383.30 and 1320.60).cm^{-1} were assigned to the C-N, or NH bending vibrations or CH_3 in the amide group (Ramya *et al*., 2012). Also, 1103 cm-1 and 600 cm^{-1} peaks were assigned to the C–O–C group (glycosidic linkage) and C-C rings, respectively.

Therefore, the CC and CPS FTIR spectra exhibited similar bands, suggesting that the two chitosan samples share identical chemical compositions.

CONCLUSION

This research successfully extracted chitosan from the local periwinkle shell and characterized it alongside commercial chitosan using FTIR. The FTIR results of both chitosan showed similar bands, suggesting they share similar chemical compositions. The proximate and properties analysis on the CPS showed favourable characteristics with a high degree of acetylation and percentage yield compared to the commercial chitosan. Hence, the chitosan prepared from the local periwinkle shell found in Akwa Ibom can be utilized to produce chitosan needed for biomedical applications, wastewater treatment and industrial applications. This will further boost the local economy and also reduce environmental hazards.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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