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EXTRACTION AND CHARACTERIZATION OF CHITIN AND CHITOSAN BIOPOLYMER AS WOUND HEALING MATERIAL USING SHRIMP SHELLS

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ABSTRACT

Wound refers to any damage or disruption to the normal anatomical structure. In our everyday pathology, wounds remains one of the demanding clinical problems with it related complications which increases mortality and morbidity rate yearly. In this study, chitin and chitosan were extracted from shrimp shells waste by chemical method, treated with an acid and an alkali which gave a yield of 12 % and 66.57 % respectively. The chemical extraction method included demineralization where the sample was treated with hydrochloric acid (HCL) under a specific temperature. The sample was then treated with sodium hydroxide (NaOH) to remove all the protein in the material at a specific temperature. To obtain the chitosan, the chitin was treated with 50% NaOH at a temperature of 1000*C*. *E.coli* proved to be more susceptible in antimicrobial studies with a value of 11.67± 0.47. The FTIR spectra gave a characteristic bands of –NH at 3430.09 cm-^{1}, OH at 3256.32 cm^{-1}.At 2960.75 cm^{-1}, NH was attached to a single bond. The characteristics of produced chitosan were in accordance with the commercial standard that showed a higher percentile yield posing many properties of commercial value and greater scope of industrial applications. This study revealed that shrimp shell waste could be effectively utilized for the extraction of chitin, chitosan, and chitooligomer for industrial applications. The zone of inhibition study of *E.coli* shows that chitosan and COS may have a high antimicrobial property hence it usefulness in the wound healing management.

Contribution/Originality: This study aimed to seek out the biomaterial present in shrimp shells waste known as chitin and chitosan biopolymers that are naturally abundant, their characterization and antimicrobial study for use in wound healing management.

1. INTRODUCTION

In recent years, injuries have been something that many people encounter but as time went by, different varieties of wound healing material have been introduced into the pharmaceutical industries to speed up healing process in most countries. In our everyday pathology, wounds remains one of the demanding clinical problems with it related complications which increases mortality and morbidity rate yearly (Alonso, Lee, Burgess, & Browner, 1996); (Natarajan, Williamson, Stiltz, & Harding, 2000). New therapeutic approaches have been introduced into the

system which emphasis more on injury cure management (Velnar, Bailey, & Smrkolj, 2009). Anitha and others said that bio-polymers have been investigated and used in different pharmaceutical and biomedical industries for several years (Jayakumar et al., 2011). The multifunctional behavior of bio-polymers facilitate their application to a wide variety of wound types. These bio-polymers are polymers that are found in nature and an example is chitin and chitosan which is the second most abundant bio-polymer. The role of chitin and chitosan as biomaterials are amazing as evidenced by the published scientific papers and patents. Chitin and chitosan are attracting increasingly more attention recently due to its biological and physicochemical characteristics. Chitin and chitosan with beneficial biological and antimicrobial properties and high valuable potential for wound healing are attractive for wound care. Healing restores integrity of the injured tissue and prevents organisms from deregulation of homeostasis. Wound treatment have gradually developed from the olden days. Dressing material for wound application was aimed at inhibiting bleeding, protecting the wound from any unusual environmental irritants as well as disturbances of water and electrolyte. Skin plays an important role in homeostasis and the prevention of invasion by microorganisms (Suzuki, Matsuda, Isshiki, Tamada, & Ikada, 1990). The skin generally needs to be covered with a dressing immediately after it was damaged. Huge amount of chitin is contained in shrimp shell which serves as an expensive ingredients used in many cosmetics, pharmaceutical and food products. Researchers have focused on biologic-synthetic dressings. This is because biologic-synthetic dressings are bilayer and consist of high polymer and biologic materials. These three categories of wound dressing which are biologic, synthetic and biologic-synthetic wound dressing are all used frequently in the clinical setting, but none is without disadvantages. An ideal dressing should maintain a moist environment at the wound interface, allow gaseous exchange, act as a barrier to microorganisms and remove excess exudates (Singh, Shitiz, & Singh, 2017). Chitin and chitosan are examples of bio-polymers which are biodegradable, non-toxic, and bio-compatible and has antimicrobial and homeostatic activity. These bio-polymers promote growth and facilitate wound healing (Kaya et al., 2016).

The isolation of chitin and chitosan from shrimps shell was successfully done using the chemical method. The extracted chitosan, chitooligosaccharide (COS) and chitin was characterized using Fourier Transform Infrared spectroscope. The antimicrobial studies on chitosan, chitooligosaccharide and chitin was performed using the Mueller Hinton agar plate using the *Escherichia coli* (*E.coli*), *Staphylococcus aureus* (*S. aureus*), *Candida albican* (*C. albican)* and *Aspergillus niger* (*A. niger)*. Finally a biocompatible product was developed successfully using the chitosan

2. MATERIALS AND METHODS

2.1. Collection of Shrimp shells

This study was carried out in the Phyto-chemistry laboratory of the Center of Plant Medicine Research, Mampong (Ghana) and in collaboration with the Department of Biomedical Engineering, All Nations University-Ghana, in the month of June 2020 to August 2021. The fresh shrimp shells were collected from the Atinpoku River in the Eastern region of Ghana washed, aired and oven dried at 60^0 C for three days. The dried shells was then grinded into fine particles for further studies.

2.2. Extraction of Chitin and Chitosan

A 90g fine particle of shrimp shell was extracted with 316.5 ml of 2M HCL at room temperature for 24 hours at 80^0 C. The demineralized content was washed with distilled water repeatedly until the pH became neutral and the residue obtained was dried at 50^0 C for nine (9) hours. The demineralized shells were deproteinized at room temperature for twenty (20) hours by treating it with 150 ml of 2M NAOH at 100^0 C and sample filtered, drained off and washed several times with distilled water and then oven dried at 50^0 C for 5 hours 30 minutes. The sample was treated with 100 ml acetone with vigorous stirring for four (4) times repeatedly at an interval of 30 minutes to remove its pink coloration and was further oven dried at 50^0 C. The decolorized chitin was deacetylated by

extracting with 50% concentrated NAOH at 100^{0}C for 5 hours in a solid to solution ratio 1:10 (w/v) to form the chitosan. The alkali was drained off, washed repeatedly with distilled water until it pH was lowered. Chitosan was dried at 50^0 C for five (5) hours and it was later stored for further analysis (Bölgen, Demir, Öfkeli, & Ceylan, 2016).

2.3. Calculation of Percentage Yield of Extracted Chitin and Chitosan

The percentile yield of the chitin and chitosan extracts were weighed and calculated by dividing the weight of the extracted chitin by the weight of chitosan isolated in grams to the initial dry shrimp shell weight by the dry chitin weighed before deacetylation respectively. The yields were calculated as follows;

Yields of chitin (%) = [Extracted chitin (g)/shrimp shells (g)*100].

Yield of chitosan (%) = [isolated chitosan (g)/chitin (g)*100].

2.4. Characterization of Extracted Chitin and Chitosan

The chitosan extracted from the shrimp shell was characterized using Fourier Transform Infrared Spectroscopy (FTIR) for the analysis of the degree of light interference of the extracted chitosan.

2.5. Antimicrobial Susceptibility Test

The Mueller-Hinton agar was prepared and poured into a petri plate and a 100µl inoculum was spread gently using a sterile cotton swab stick. A cork borer of about six (6) millimeter diameter was used to create six (6) different wells and 80µl of the concentration of chitin, chitosan and COS were impregnated into the well. The petri plate was allowed to stand for two (2) hours for better diffuse and then incubated overnight at different temperatures depending on the organism used. Bacteria's such as *Staphylococcus aureus* (*S.aureus*), *Candida albican* and *Escherichia coli (E.coli)* were incubated at 35^0 C for twenty four (24) hours whiles *Aspergillus niger* is incubated at 25^0 C for three (3) days . The maximum zones of inhibition were measured in millimeters using a rule.

3. RESULTS AND DISCUSSION

As shown in the figure below are the stages of the sample preparation showing the raw shrimp shells of freshly washed shrimps that has been air dried. The Figure 1A depicts shrimps after it has been dried in the oven, Figure 1B shows the shells neatly removed from the shrimps and Figure 1C shows shrimp shells grinded into fine particle to be used for further analysis.

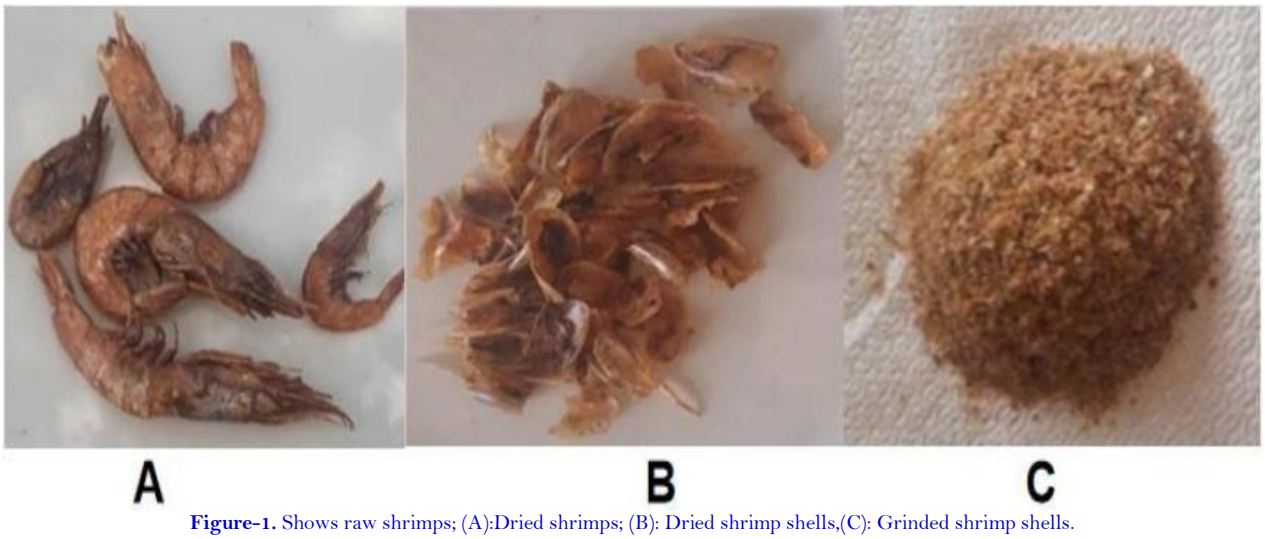

Figure-1. Shows raw shrimps; (A):Dried shrimps; (B): Dried shrimp shells,(C): Grinded shrimp shells.

The demineralization and deproteinization process was performed using the powdered shrimp shells. As shown in Figure 2 below, the Figure 2A shows the grinded shrimp shells in 2M HCL heated at 80^0 C for five and a half hours. The Figure 2B shows repeated washing phase of the grinded shrimp shells during decantation method. And Figures 2C and 2D respectively shows grinded shrimp shells after filtration and then oven dried. The sample was packed for further studies.

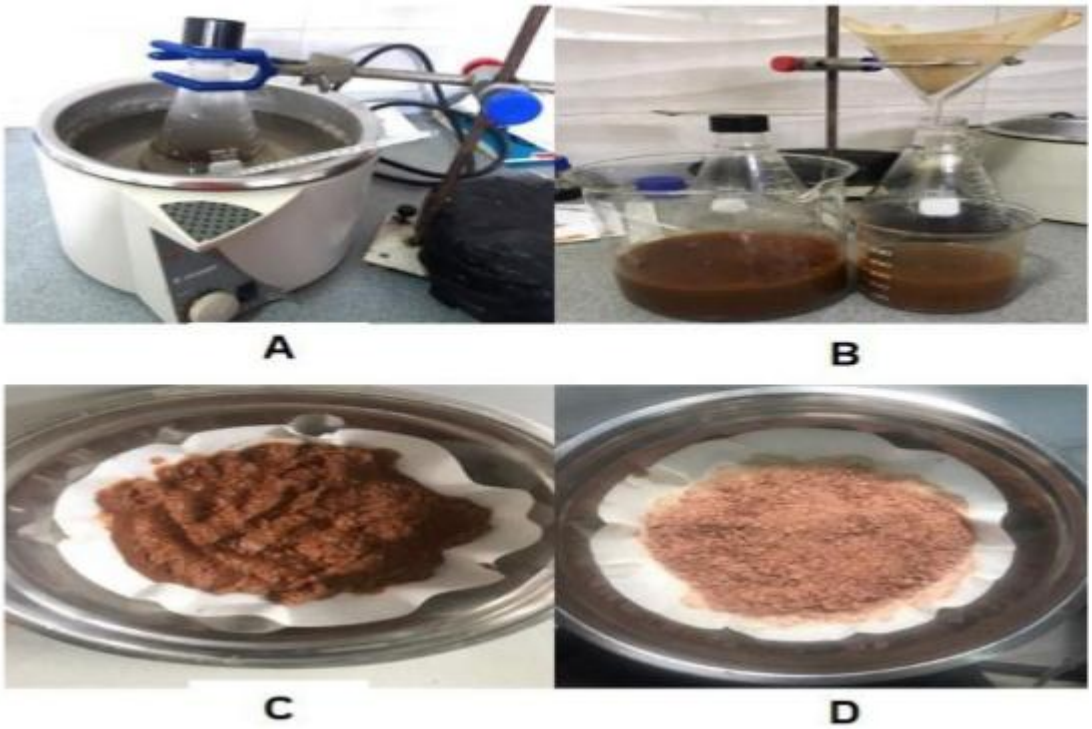

Figure-2. Shows powdered shrimp shells during the demineralization stage (A): Powdered shrimp shell in 2 M HCL; (B): Decantation of powdered shrimp shells; (C): filtered powered shrimp shells after decantation; (D): powdered shrimp shells after hot oven dry.

The deproteinization procedure was carried out successfully to obtain the final chitin for the further analysis of the study. This was achieving when the obtained sample was treated with 2M NAOH. The Figure 3A depict the dried sample after it was oven dried for five and a half hour to finally obtained the whitish chitosan from the extracted chitin in Figure 3B using shrimp shells as shown in Figure 3C.

Figure-3. Shows the deproteinization procedure: **(A):** Dried Sample after deproteinization; **(B):** extracted Chitin and **(C):** Extracted chitosan.

3.1. Calculation of Percentage Yield of Extracted Chitin and Chitosan

The yield of extracted chitin from the shrimp shells was 12%. The yield of chitosan produced from extracted chitin was 66.57%, which was similar to the results that was reported in the literature by Kaya *et al.* of 76% yield of chitosan from *Callinectes sapidus* and Oduor *et al.* reported 74.6% yield of chitosan of *Sylla cerrata* (Bölgen et al., 2016; Oduor, Struszczyk, & Peter, 2008). The yields were above average in these studies and our study indicated that shrimps are one of the major resources of chitin and chitosan among the other crustacean group of organisms.

3.2. Characterization of Chitosan using FTIR

The FTIR study depicted that the chitosan was extracted successfully. The Figure 4 below demonstrates the FTIR spectra of the shrimp shell chitosan extracted. The FTIR spectra shows the characteristic bands of –NH at 3430.09 cm^{-1} and carbonyl group band at 3256.32 cm^{-1}. The band at 2960.75 cm^{-1} could be assigned to NH attached to a single bond. Any band which comes after 3000 cm-1 is a double bond attached to the amine group. The band at 3098.19 cm^{-1} is associated with (N-H) in secondary amides only with trans-configuration and usually is due to the formation of linear associates. Usually, at peak 1750-1800 cm^{-1} there is C = O. The figure had no peak within that range.

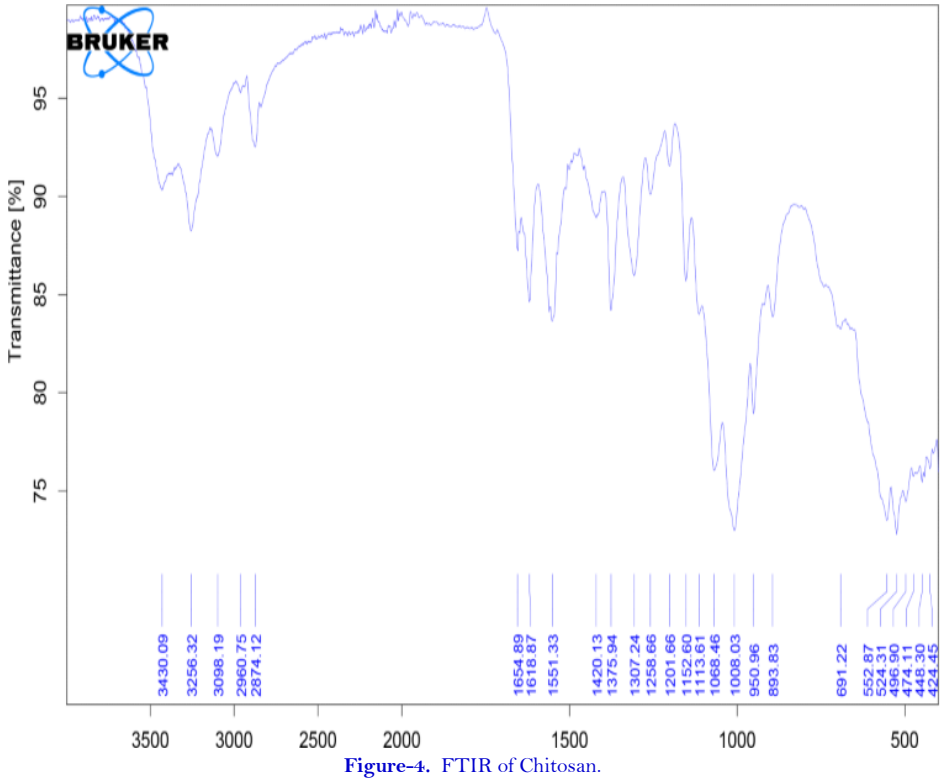

Figure-4. FTIR of Chitosan.

3.3. The Maximum Inhibition zone of the Antimicrobial Studies

The anti-microbial study was done successfully using four different microorganisms during the study. After 24 hours of culturing, the maximum inhibition zone were observed and measured as tabulated in Table 1.

The well diffusion method were used throughout the study. Mueller-Hinton (M-H) agar was prepared and poured into a conical flasks for autoclave as per number of bacteria under studied and poured into a sterilized petri plates. After solidification of the agar, wells were punched by sterile borer aseptically under laminar airflow, kept overnight in an incubator for the growth of zone of inhibition to occur. Afterward, the zone of inhibition was

measured. The test was done in triplicates. However, among the microorganism used *E.coli* proved to be more susceptible.

Table-1. Table of measured zone of inhibition. (* means the mean and standard deviation values calculated).

	Chitin*	Chitosan*	COS*
E.coli	6 ± 0	6± 0	11.67 ± 0.47
S.aureus	6 ± 0	6 ±0	6 ±0
C. albican	6 ± 0	6 ±0	6 ±0
A.niger	6 ± 0	6± 0	6± 0

The table above indicates the zone of inhibition for all the four (4) species of microorganisms used. The zone of inhibition for *E.coli*, *S.aureus*, *C.albican* and *A.niger* in the case of chitin and chitosan were 6 ± 0 which indicates that these species had no growth after incubation. *E.coli* used on COS had a growth of 11.67 ± 0.47 which when compared to the reference standard 18.33 ± 0.47 indicates that it can be used in different management of wound care.

4. CONCLUSION

Chitosan was extracted using modified method of previous studies. The characteristics of produced chitosan were in accordance with the commercial standard that showed a higher percentile yield posing many properties of commercial value and greater scope of industrial applications. This study revealed that shrimp shell waste could be effectively utilized for the extraction of chitin, chitosan, and chitooligomer for industrial applications. Chitin was extracted from the waste of shrimp shells and chitosan was produced from extracted chitin by deacetylation. The chemical composition of shrimp shell and the extracted chitosan was characterized using FTIR. In addition to this, antimicrobial properties of obtained chitosan was determined by the well diffusion method as the maximum inhibition zone measured indicated that chitosan as well as COS extracted have a strong antimicrobial property hence it usefulness in the wound healing management.

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