*Chemical Science International Journal*

***30(2): 50-62, 2021; Article no.CSIJ.67628***
***ISSN: 2456-706X***
*(Past name: American Chemical Science Journal, Past ISSN: 2249-0205)*

# Extraction and Characterization of Silver Nitrate Modified Biopolymer and its Antimicrobial Mode of Action

**Jeje O. Ayorinde[1*] and Osula J. Eghosa[1]**

*[1]Department of Basic Sciences, Federal Polytechnic Auchi, Edo State Nigeria.*

***Authors' contributions***

*This work was carried out in collaboration among all authors. Author JOA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author OJE and JOA managed the analyses of the study. Author JOA managed the literature searches. All authors read and approved the final manuscript.*

***Article Information***

DOI: 10.9734/CSJI/2021/v30i230218
Editor(s):
(1) Prof. Akmal S. Gaballa, Zagazig University, Egypt..
Reviewers:
(1) Salah Raza Saeed, Charmo University, Iraq.
(2) Sara Hooshmand, Mahshad University of Medical Sciences (MUMS), Iran.
Complete Peer review History: http://www.sdiarticle4.com/review-history/67628

***Original Research Article***

***Received 01 April 2021***
***Accepted 20 April 2021***
***Published 08 May 2021***

## ABSTRACT

Biopolymer (chitosan) was isolated from crab shell waste through the processes of demineralization, deproteinization, decolourization and deacetylation. The resulting chitosan (CHS) was further treated with silver nitrate ($AgNO_3$) solution at various concentrations (0.5, 1.0 and 1.5 M) in order to enhance the antimicrobial activity of chitosan. The crab shell powder (CSP) and (CHS) were characterized using X-ray Diffractometer (XRD), Fourier Transform Infrared (FT-IR), and Scanning Electron Microscopy coupled with Energy Dispersive Spectroscopy (SEM-EDS). The antimicrobial mode of action of $AgNO_3$ treated chitosan was performed using serial dilution (1:2) technique for minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) and tested against four microorganisms (*Escherichia coli*, *Klebsiella pneumonia*, *Staphlococcus aureus* and *Pseudopodium*). The result of proximate analysis of CHS and chitin (CHN) showed % crude protein to be 12.24±001 and 20.54±0.03 respectively, % ash was found to be 4.1±0.01 for CHS and 3.80±0.01 for CHN. The FTIR spectra of CHS and CHN showed their characteristic absorption peaks and the diffractograms of CSP and CHS showed $CaCO_3$ to be the major mineral present in the samples. The antimicrobial evaluation revealed that untreated chitosan extract (UCHSE)

**Corresponding author: E-mail: jejeayorindeoludiya@gmail.com, jaminjeje@gmail.com;*

showed no antimicrobial activity against the four tested microorganisms. The results of MIC and MBC showed that the organisms responded to the antimicrobial agent at different dilution concentration. It was observed that CHS treated with 0.5 M $AgNO_3$ (0.5 SNCHSE) inhibited the growth of *E. coli* at 1000 µg/mL, *S. aureus* at 500 µg/mL while it exhibited bactericidal (MBC) activity against all the organisms at 1000 µg/mL.

*Keywords: Chitosan; microorganisms; crab shell; silver nitrate; extraction.*

## 1. INTRODUCTION

Antibacterial are chemical substances derived from or produced by a bacterium that inhibit the growth, kill microorganism and cure bacterial infections without causing any damage to the host cells. The investigation and determination of the sensitivity of pathogens to antibacterial is necessary for prescribing or selection of the most appropriate one for treating microbial infections. The antibacterial that kills bacteria are said to be bactericidal while those that prevent only their multiplication are referred to as bacteriostatic though some can both be bactericidal and bacteriostatic depending on their concentration. The dilution method is appropriate in the determination of minimum inhibitory concentration (MIC) which is the least concentration of antimicrobial agent that inhibits the growth of bacteria and the determination of minimum bactericidal concentration (MBC) which is the least concentration of antimicrobial agent needed or required to kill microorganisms [1]. The continuous increase of antibacterial resistance as a result of insufficiency of new antibiotics in the clinical institutions call for an urgent attention for the development of potent antimicrobial agents.

The antibacterial effects of silver (Ag) salts have been known for more than 2000 years but only put to use as an antimicrobial agent since the $19^{th}$ century [2]. Silver salts are now use to inhibit or slow down the growth of microorganisms in variety of applications such as, dental work, catheters, and burn wounds [2].

Silver ion ($Ag^+$) and silver-modified compounds are extremely toxic to microorganisms; they show strong biocidal activity against as many species of bacteria which include *E. coli* [3].

Gram positive bacteria have been identified as a major cause of infections and are mounting serious resistance to many antibacterial agents [4]. Lui et al. [5] have reported that dilute solutions of $AgNO_3$ were used to treat infections caused by bacterial in the $19^{th}$ century. Ewald et al., [6] have also reported that silver modified antimicrobials can be effective in the treatment of infections on account of non-toxicity of active silver ion ($Ag^+$) to human cells. Previous researches have studied the antibacterial potential of silver (Ag) modified material on both gram-positive and gram-negative bacteria such as *S. aureus*, *E.coli*, *Pseudononis aeruginosa* and *Staphlococcus epidermid* [7,8].

Chitosan is a natural biopolymer that made up of copolymers of glucosamine and N-acetyl glucosamine. Chitosan is extracted by partial deacetylation of chitin which is the main structural unit of crustacean and cell walls of some fungi [9,10]. The mode of action of chitosan is based on the disruption of cell membrame [11], inhibition of RNA and protein synthesis, distruption of intracellular cell components [12], ionic interaction [13] and the presence of amino groups in the structure of chitosan enhances its antimicrobial activity [14]. [15] Jianhui et al., have reported that cationic formation based on the protonation of amino group also contributes to the death of microorganisms.

Enzymatic or alkali method has been used to convert chitin into chitosan but alkali method has been the most used method. The main aims and objectives of this study therefore, are to extract biopolymer (chitosan) from crab shell waste, determine their physicochemical properties, modify chitosan with various concentration of silver nitrate solution and investigate their antimicrobial activity against four different microorganisms.

## 2. MATERIALS AND METHODS

### 2.1 Sample Collection and Materials/ Reagent Used

The crab shells were collected at Pessu market in Warri Delta State, Nigeria. All the reagents used were of analytical grade.

### 2.2 Test Microorganisms

The antimicrobial activity of extract from chitosan and silver nitrate modified chitosan was

investigated against four strains of bacteria (*Escherichia coli*, *Klebsiella pneumonia*, *Staphlococcus aureus* and *Pseudopodium*). The inocula were prepared and cultured in Auchi Polytechnic Cottage Hospital Laboratory.

## 2.3 Preparation of Crab Shells

The Crab shells were thoroughly washed with hot water to remove the surface dirts and the remaining fleshy materials. The shells were air dried for five days and ground to powder using milling machine. In order to obtain the desired particle size, the sieves were nested in descending aperture size from top to bottom (300, 250, 200, 150, 100 nm). The particle size of 100 nm was selected and used in the study.

## 2.4 Demineralization of the Crab Shell Powder (CSP)

CSP (150 g) was weighed, transferred into 1000 mL beaker, and 500 mL of 1 M HCl was added and stirred thoroughly. The mixture was soaked at ambient temperature for 24 hr, filtered and the residue was washed severally with distilled water until neutral pH was attained.

## 2.5 Deproteinization of CSP

The demineralized CSP (84.33 g) was transferred into 1000 mL beaker and 500 mL of 1.5 M NaOH solution was added at ambient temperature, allowed to stand for 48 hr and stirred intermittently. The mixture was washed with distilled water to remove residual alkali solution until neutrality was attained. The resulting residue (chitin) was dried in an oven, weighed and labeled as CHN.

## 2.6 Decolouration of CHN

The deprotenized sample (chitin) was transferred into a 1000 mL beaker and 300 mL of sodium hypochlorite (NaClO) solution was added to decolourize it. The mixture was initially separated by decantation followed by filtration.

## 2.7 Deacetylation of CHN

The resulting CHN was weighed into a beaker and 9.5 M NaOH (1:10 w/v) was added and stirred thoroughly for 48 hr at ambient temperature and then filtered. The filtrate was washed continuously with distilled water to remove the residual alkali until neutrality, oven dried at 105 °C for 2 hr, stored in a dry container, the solid (chitosan), was labeled (CHS).

## 2.8 Modification of CHS using $AgNO_3$

The CHS (1.5 g) was weighed into 250 mL beaker and 50 mL of 0.5 M ($AgNO_3$) solution was added and mixed thoroughly. The mixture was allowed to stand for 23 hr and heated in a thermostatically controlled hot plate at 80 $^{O}$C for 30 min, filtered and dried in an oven at 105 $^{o}$C for 1 hr and labeled as 0.5 SNCHS. The process was repeated for 1 M and 1.5 M $AgNO_3$ and labeled as 1.0 and 1.5 SNCHS respectively while the untreated CHS was labeled UCHS.

### 2.8.1 Extraction procedure

The CHS (1.0 g) was weighed into a beaker and 100 mL of 1 % acetic acid was added. The mixture was allowed to stand for 11 hr and filtered. The resulting filtrate was collected and stored in an amber sample bottle labeled untreated chitosan extract (UCHSE). The same procedure was repeated for other concentrations 0.5, 1.0 and 1.5 M and labeled as 0.5, 1.0, and 1.5 SNCHSE respectively stored and used for the antimicrobial analysis.

#### 2.8.2 Characterization of samples

X-ray diffraction (XRD) analysis of CSP and CHS was carried out to detect their mineral compositions and crystallinity. Miniflex. 600, Rigaku corporation Japan machine was used for the analysis. The intensity was measured at Bragg's 2θ angle. All samples surface morphology and elemental composition were observed from Scanning Electron Microscope coupled with Energy Dispersive X-ray spectroscopy (SEM-EDS) using Phenom Prox, Phenom world, Eindhoven, The Netherlands. The Fourier Transform Infrared (FTIR) spectra of CHS and CHN were obtained over a range of 4000-350 $cm^{-1}$ using Perkin-Elemer spectrum-2 spectrophotometer and the spectrum was recorded using attenuated total reflectance (ATR). The adsorption in the infrared region occurred as a result of rotational and vibrational movement of the molecular groups and chemical bond of a molecule [16].

## 2.9 Proximate Analysis CHS and CHN

Proximate analysis of CHS and CHN extracted from crab shells were carried out to determine crude protein, fibre content, ash content, ether

extract, crude fibre, dry matter, ME(kcal/kg) and carbohydrate (CHO). They were determined by standard method as described by AOAC [17].

## 2.10 Antibacterial Determination

### 2.10.1 Determination of minimum inhibitory concentration (MIC) and minimum bactricidal concentration (MBC)

Minimum inhibitory concentration was carried out by a serial dilution (1:2) technique with five different test tubes. Serial dilution of concentration 1000, 500, 250, 125, and 62.5 µg/mL were prepared from UCHSE and (1.0 µg/mL) was measured and transferred into a test tube and inoculated with the test organisms. The same procedure was performed for 0.5, 1.0, 1.5 SNCHSE and positive control. The cultures were incubated at 37$^{o}$C for 24 hr. the tubes were inspected visually to determine the growth as indicated by turbidity, in fact turbidity of the cultured medium is an indication of the presence of a large number of microbes and the tubes in which the antibacterial agent is present in concentration large enough to inhibit bacteria growth remain clear and is regarded as the minimum inhibitory concentration (MIC). The minimum bactericidal concentration (MBC) was determined by inoculating the suspension from each test tube in BHI agar plate which showed no bacteria growth after incubation was seeded in agar plates and were then incubated at 37$^{o}$C for 24 hr. The lowest concentration of the modified sample extract at different concentrations that kill the initial bacteria population indicating no colony on the agar after 24 hr of incubation at 37$^{o}$C was recorded as the minimum bactericidal concentration. The presence or absence of turbidity was denoted as negative (–) and positive (+) respectively.

# 3. RESULTS AND DISCUSSION

## 3.1 Elemental Composition of CSP and CHS

The elemental composition of CSP and CHS was compared as presented in Table 1. This is to ascertain the degree of demineralization carried out on the CSP compare to CHS that was extracted from CSP. The weight percent of the elemental composition of CSP and CHS in the table reveals that the concentration of the native elements decrease after demineralization of CSP using 1 M HCl and the major element that is abundant in CSP and CHS is calcium (Ca) (weight%, 70.9 and 61.32 respectively). However, other elements are available in little quantity.

## 3.2 Scanning Electron Microscopy (SEM) of CHS and CSP

Plate 1 (a) and (b) are the surface morphology of CHS and CPS. The plates revealed the changes in their surface structure. It is observed that some impurities and particle have been leached from CPS leading to the smooth surface of CHS and also more pore volume and sizes have been created compare to CPS where there is agglomerate of particles with few vacuoles in between the particles.

## 3.3 X- Ray Diffraction Analysis of CSP and CHS

In order to understand the changes that have occurred in the mineral composition and crystallinity of CSP and CHS the use of X-ray diffractometer was used to observe the changes. The X-ray Diffraction (XRD) is an instrument used for the identification of crystalline phases of inorganic compounds. The diffractogram obtained from the XRD as shown in Figs. 1 and 2 Show the characteristic mixture of crystalline, amorphous solid particles in CHS and CPS and their respective mineral compositions. It is observed that the major mineral in CHS and CSP is calcite with the peak at 2θ values of 29$^{o}$ but the arrangement of particles in CPS is more orderly, crystalline than CHS due to the appearance of strong and sharp peak in CPS diffractogram. The peaks are a characteristic pattern of $CaCO_3$. Other minerals present are chlorapatite, quartz, lime and dolomite. The sharper peak is an evidence of denser crystalline structure observed in CSP while CHS shows mixture of both crystalline and amorphous structure.

## 3.4 Proximate Analysis of CHS and CHN

The proximate analysis result is presented in Table 2, the result shows that dry matter value obtained for CHN was observed to be lower than the corresponding CHS. This could be attributed to the drying procedure in which CHN was subjected to before its deacetylation to yield CHS and probably CHS absorbed moisture on exposure or water molecules were trapped in the crystal lattice of CHS and the amount of water can be affected by atmospheric conditions in the

laboratory after the sample bottle is opened [18]. Korea Food Additive Code (KFDA) in [19] gave a report that moisture content of CHS powder should be lower than 10 % and from this study it shows that CHS (7.03±0.04) is of acceptable quality. Nessa et al., [20] have reported that a high-quality grade of chitin and chitosan should contain ash content less than 1 %. Ash is the inorganic residue remaining after organic matter and moisture have been removed from a sample. The ash content of CHN in this study was lower in value than that of CHS as it could be due to the presence of acetyl group in the structure of CHN as earlier reported by Isa et al.,[21] CHS and CHN in this study have ash content (CHS, 4.1±0.01 and CHN, 3.80±0.01) higher than 1 % this could be due to the low concentration of mineral acid (1 M HCl) used during the demineralization stage which is also evident in the higher % CHO content of CHS (77.80±0.01) and CHN(68.83±0.72). It is also an indication of high mineral content. The crude protein of CHN was found to be higher which could be as a result of low degree of deacetylation of CHN (it contains more nitrogenous substances than CHS). The higher fibre content recorded in CHS could be attributed to the removal of more matter from CHN during its deacetylation to yield CHS and also an indication of high content of cellulose Muhammed et al., [22].

## 3.5 FT-IR Analysis of CHS and CHN

The quality features of infrared spectroscopy have been so useful and one of the most effective tools for characterization [23] especially for functional groups and for the elucidation of the interactions and structure of chitosan (CHS) and chitin (CHN). Presented in figure 3 and 4 are the spectra of CHS and CHN. The spectra obtained reveal some structural changes in CHS and CHN. This is evident in shifting, formation, appearance and absence of certain functional groups. Most conspicuous is the absorption peak that appeared at 3697.10 $cm^{-1}$ in CHS after deacetylation of CHN. Figure 3 is the FTIR spectrum of CHS. The absorption band at 3697.10 $cm^{-1}$ is attributed to OH probably from trapped water molecule in the crystal lattice of CHS or due to absorption of moisture from the atmosphere as a result of exposure in the laboratory. The absorption peak at 3444.55 $cm^{-1}$ is assigned to N-H and OH (stretch) functional groups from amine group while the absorption peak at 3110.4 $cm^{-1}$ is due to O-H stretch. The bands that appear at 2962.7, 2926.68 and

**Table 1. Elemental composition of CSP and CHS**

| Elemental Composition of Crab shell powder and Chitosan | | |
|---|---|---|
| **Element** | **Weight %** | |
| | CSP | CHS |
| Ca | 70.9 | 61.32 |
| K | 3.39 | 2.71 |
| Cl | 3.31 | 2.63 |
| Al | 1.99 | 1.74 |
| Zn | 1.54 | 1.26 |
| Na | 1.23 | 0.73 |

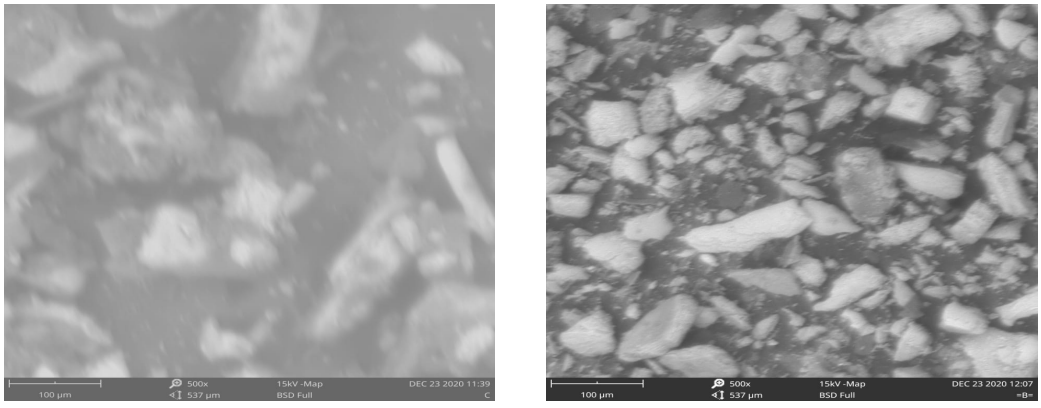

**Plate 1. Showing the surface morphology of (a) CHS and (b) CSP**

**Table 2. Proximate Analysis of CHS and CHN**

| | % Moisture | % crude protein | % ash content | % ether extract | % crude fibre | % dry matter | ME(kcal/kg) | % CHO |
|---|---|---|---|---|---|---|---|---|
| CHS | 7.03±0.04 | 12.24±0.01 | 4.1±0.01 | 2.48±0.04 | 3.40±0.01 | 92.94±0.01 | 3414.55±1.43 | 77.80±0.01 |
| CHN | 6.82±0.04 | 20.54±0.03 | 3.80±0.01 | 3.06±0.06 | 3.20±0.01 | 92.83±0.46 | 3474.82±1.49 | 68.83±0.72 |

*The values presented in the table represent the mean ± SD of two replicates*

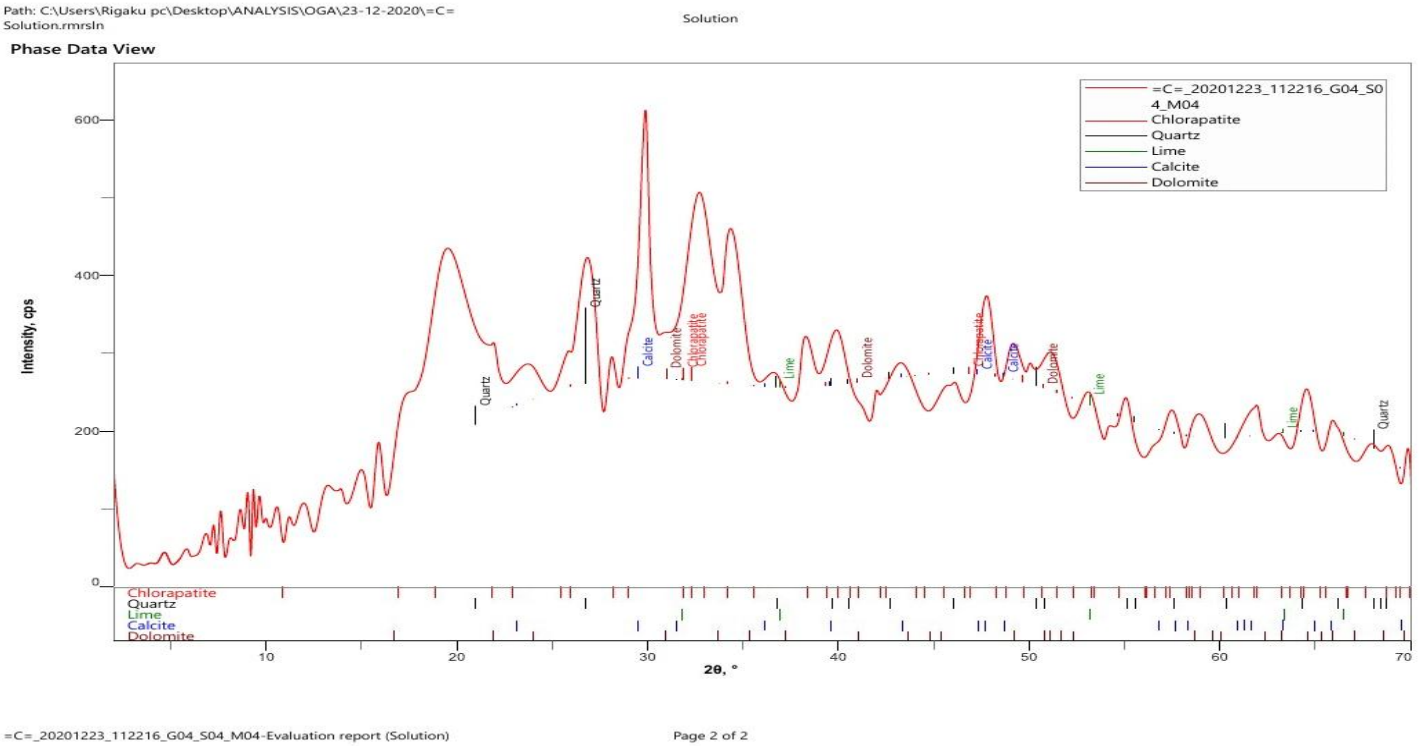


**Fig. 1. X-ray diffraction diffractogram of CHS**

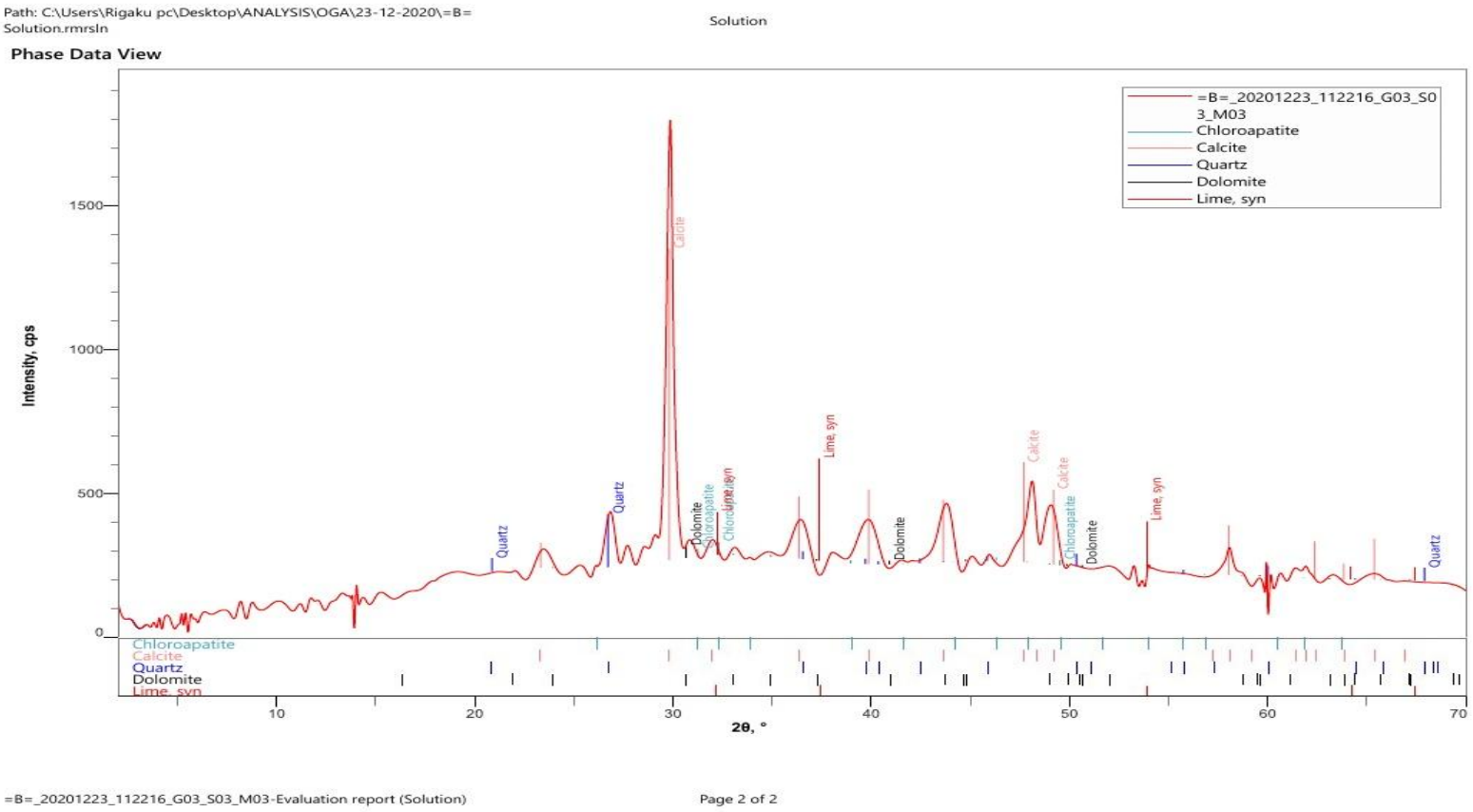


**Fig. 2. X-ray diffraction diffractogram of CSP**

2892.3 $cm^{-1}$ were as a result of C-H functional group from alkyl group in CHS while the absorption band at 1659.97 $cm^{-1}$ is attributed to C=O in $2^o$ amide, however, the peaks at 1557.02 and 1423.00 $cm^{-1}$ were assigned to C=O in NHCOCH3 amide I and $NH_2$ in $NHCOCH_3$ amide II respectively whereas the band at 1380.24 $cm^{-1}$ is due to $CH_3$ in $NHCOCH_3$ and the appearance of absorption peak at 1423.00 $cm^{-1}$ is due to $CH_2$ in $CH_2OH$ group. The FTIR spectrum of CHS shows the formation of bond at 1317.58 $cm^{-1}$ which is attributed to C-H in pyranose and an absorption band at 1251.50 $cm^{-1}$ indicating the presence of C-N due to complex vibrations of NHCO group (amide III). The peak that appears at 1156.93 and 1073.96 $cm^{-1}$ are attributed to the presence of C-O-C functional group (glycosidic linkage). The absorption peaks at 1028.27 and 953.38 $cm^{-1}$ are assigned to C-O in secondary O-H group and CO in primary O-H group amide III respectively while the peak at 872.96 $cm^{-1}$ is assigned to the presence of calcite in the sample.

Presented in figure 4 is the FTIR spectrum of CHN. The presence of absorption band at 3444.09 $cm^{-1}$ is attributed to $OH/NH_2$ group while the absorption peak that appears at 3110.4 $cm^{-1}$ is due to O-H stretch while the peaks that appear at 2962.7 and 2929.75 $cm^{-1}$ are assigned to CH from alkyl group. The characteristic absorption band at 1659.15 $cm^{-1}$ is due to $2^o$ amide and the band at 1567.77 $cm^{-1}$ is as a result of the presence of $NH_2$ from $NHCOCH_3$ is attributed to CH in pyranose. However, the absorption band at 1261.54 $cm^{-1}$ indicates the presence of CN complex vibrations of NHCO group (amide III). The absorption peak at 1156.77 and 1074.32 $cm^{-1}$ show the presence of C-O-C glycosidic linkage and the absorption peaks at 1027.45 and 953.27 $cm^{-1}$ are due to C-O in $2^o$ OH group and $1^o$ OH group respectively while the peak at 896.51 $cm^{-1}$ is an indication of the presence of O-H group; CaO absorption band is noticed at 450.62 $cm^{-1}$. However, other absorption bands such as those that appear at 466.34, 432.09 and 413.16 $cm^{-1}$ are noticed.

## 3.6 Antimicrobial Analysis

The antimicrobial mechanism of CHS depends on the host microbes as reported by Másson et al., [24] and the difference in composition of membranes and cell walls of microbes also contribute to their antimicrobial mode of inhibiting the growth of microbes [25]. Viegas et al., [26] have also reported that electrostatic interactions could be the main factor responsible for the antibacterial activity of chitosan. The result obtained for the MIC and MBC investigation as shown in Table 3 reveals that no turbidity was observed in the test tubes 1000, 500, 250 µg/mL containing UCHSE indicating the growth of microbes. This suggests that UCHSE has no antibacterial effect against the strain of the four tested microorganisms. It is therefore obvious that the organisms are resistant to the achievable UCHSE level administered in this study and therefore no MIC and MBC were achieved. The antimicrobial activity of chitosan is affected by different factors which includes viscosity, molecular weight [5,27], degree of polymerization [28,29] and degree of deacetylation [30]. Molecular weight and viscosity of chitosan affect the diffusion of samples in Mueller Hinton Agar media in the antimicrobial activity assay. The poor antimicrobial activity of UCHSE (unmodified chitosan) could therefore be attributed to low degree of de-acetylation, high molecular weight and high viscosity. Antibacterial potential of CHS is also a function of the origin which could also be as a result of the inability of UCHSE to obstruct the growth of the microbes however, Hadi et al., [31] have reported the inability of CHS to inhibit the growth of *S. aureus* species.

The result of the test tube of 0.5 SNCHSE as observe in Table 4 shows formation of colony (growth of microbes) was noticed in test tube (1000 µg/mL) in which *S. aureus* showed resistance against 0.5 SNCHSE while in concentration of 500 µg/mL, no turbidity (no visible growth of microbes) was observed exhibiting inhibition of microbial growth, an indication of antimicrobial activity of 0.5 SNCHSE against *S. aureus*. This is an indication of 0.5 SNCHSE at the surface of the cell to form a polymeric membrane which obstruct nutrients from penetrating the cell membrane [32]. However, in concentration of 250 µg/mL, turbidity (colony, visible growth of microbes) was observed in the tubes showing that all the tested organisms showed resistance to the antimicrobial agent (0.5 SNCHSE) at that concentration. The MBC results show that at concentration of 1000 µg/mL no growth was observed in all the inoculated test tubes confirming it bactericidal effect. This shows that 0.5 SNCHSE at 1000 µg/mL killed the microbes whereas in concentrations of 500 and 250 µg/mL, the tested organisms showed resistance to the antimicrobial agent used (0.5 SNCHSE) since growth of microbes was noticed in the tubes due to the turbidity observed in the tubes.

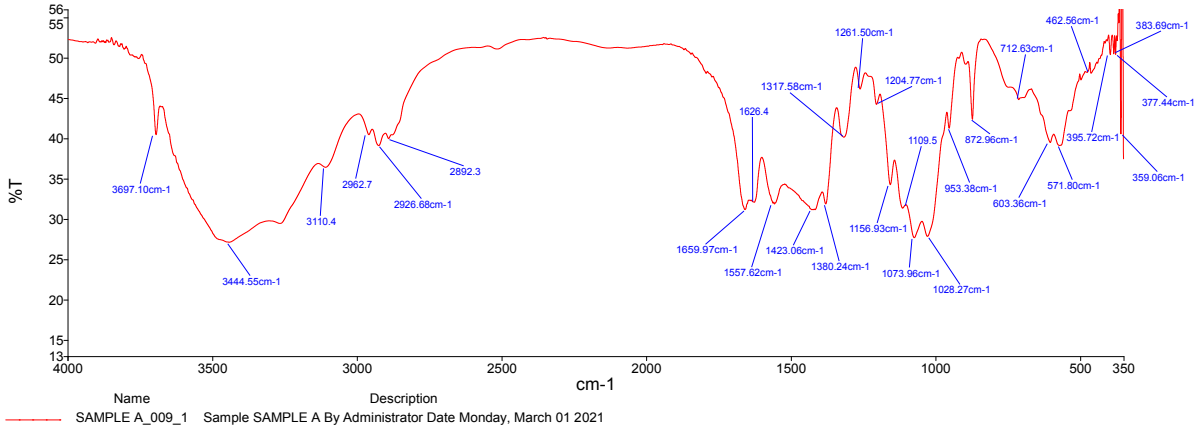


**Fig. 3. Fourier transform infrared spectrum of CHS**

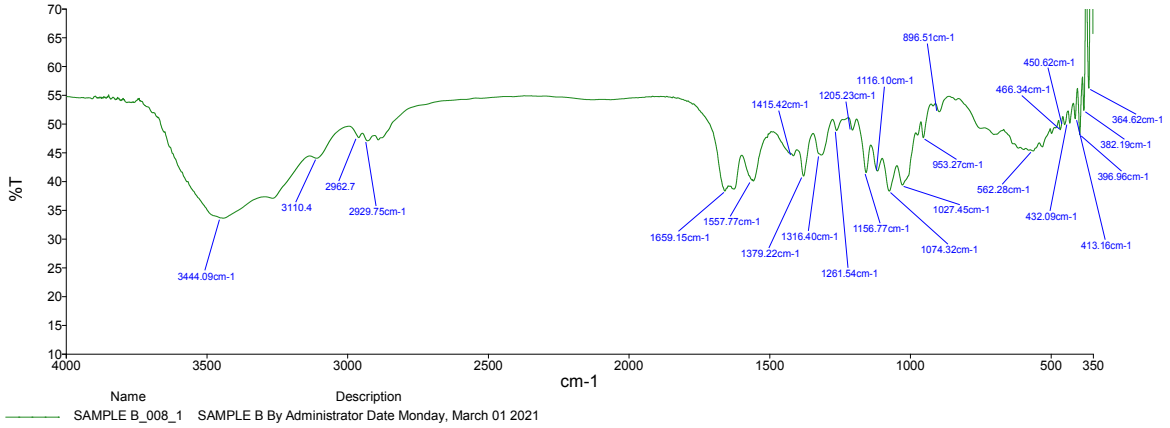


**Fig. 4. Fourier Transform Infrared spectrum of CHN**

**Table 3. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of UCHSE**

| µg/mL | *E. coli* | *S.aureus* | *K. pneumonia* | *Pseudopodium* |
|---|---|---|---|---|
| **MIC** | | | | |
| 1000 | - | - | - | - |
| 500 | - | - | - | - |
| 250 | - | - | - | - |
| **MBC** | | | | |
| 1000 | - | - | - | - |
| 500 | - | - | - | - |
| 250 | - | - | - | - |

*Negative (-) = indicate no inhibition (growth)*

**Table 4. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of 0.5 SNCHSE**

| µg/mL | *E. coli* | *S.aureus* | *K. pneumonia* | *Pseudopodium* |
|---|---|---|---|---|
| **MIC** | | | | |
| 1000 | + | - | - | + |
| 500 | - | + | - | - |
| 250 | - | - | - | - |
| **MBC** | | | | |
| 1000 | + | + | + | + |
| 500 | - | - | - | - |
| 250 | - | - | - | - |

*Positive (+) = indicate inhibition (no growth); Negative (-) = indicate no inhibition (growth)*

**Table 5. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of 1.0 SNCHSE**

| µg/mL | *E. coli* | *S.aureus* | *K. pneumonia* | *Pseudopodium* |
|---|---|---|---|---|
| **MIC** | | | | |
| 1000 | - | - | - | + |
| 500 | - | + | - | - |
| 250 | + | - | - | - |
| **MBC** | | | | |
| 1000 | - | - | - | + |
| 500 | + | + | - | - |
| 250 | - | - | - | - |

*Positive (+) = indicate inhibition (no growth); Negative (-) = indicate no inhibition (growth)*

**Table 6. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of 1.5 SNCHSE**

| µg/mL | *E. coli* | *S.aureus* | *K. pneumonia* | *Pseudopodium* |
|---|---|---|---|---|
| **MIC** | | | | |
| 1000 | - | - | + | + |
| 500 | + | - | - | - |
| 250 | - | - | - | - |
| **MBC** | | | | |
| 1000 | - | - | + | + |
| 500 | + | - | - | - |
| 250 | - | - | - | - |

*Positive (+) = indicate inhibition (no growth); Negative (-) = indicate no inhibition (growth)*

Table 5 shows the MIC and MBC of chitosan modified with 1.0 $AgNO_3$ (1.0 SNCHSE) at different concentration of serial dilution (1000, 500 250 µg/mL). no turbidity was noticed in the test tubes 1000, 500, and 250 µg/mL against *Pseudopodium*, *S. aureus* and *E. coli* respectively whereas turbidity (growth of microbes) was observed in test tube 1000 µg/mL against *E. coli*, *S.aureus* and *K. pneumonia* while in tube 500 µg/mL against *E. coli*, *K. pneumonia*, and *Pseudopodium* and in test tube 250 µg/mL against *S. aureus*, *K. pneumonia*, and *Pseudopodium*. The result obtained for MBC indicates that 1.0 SNCHSE at 1000 µg/mL showed antibacterial potential against the entire four tested microorganism except *Pseudopodium* while at 500 µg/mL the antimicrobial agent exhibit its potential against *E. coli*, and *S. aureus* but for *K. pneumonia* and *Pseudopodium* and at 250 µg/mL, it showed no antimicrobial property against the tested microorganisms. The antibacterial effect (MIC and MBC) of 1.5 SNCHSE at various serial dilution concentrations is reported in Table 6 The result reveals that 1.5 SNCHSE exhibit antibacterial effect against *K. pneumonia* and *Pseudopodium* at 1000 µg/mL and *E. coli* at 500 µg/mL an indication that 1.5 SNCHSE penetrated the cell through pervasion (Islem and Marguerite, 2015) whereas at 1000 µg/mL 1.5 SNCHSE no inhibition of of *E. coli*, *S. auerus* was observed and at 500 µg/mL, growth of microbes (*S. aureus*, *K. pneumonia* and *Pseudopodium*).was noticed. However, all the inocula showed no resistance at 250 µg/mL an indication that at low concentration of 1.5 SNCHSE no antibacterial effect was observed. The MBC results show that 1.5 SNCHSE only showed antibacterial effect against *K. pneumonia*, *Pseudopodium* at 1000 µg/mL and *E. coli* at 500 µg/mL whereas, at 250 µg/mL, no antimicrobial effect on the tested microorganisms.

## 4. CONCLUSION

Chitosan was practically extracted from crab shell waste and chitosan was modified with various concentrations of $AgNO_3$ solution to enhance its antimicrobial property. The % moisture of CHS was found to be higher than CHN while the % crude protein of CHN was higher than CHS probably due to deacetylation of chitin. The spectra of CHS and CHN showed their characteristic absorption peaks and also suggested the extraction of chitosan from chitin. XRD showed the crystalline and amorphous nature of CHS and CSP and also observed from the diffractogram that the major mineral in CHS and CSP is calcite. The MIC and MBC suggested that the treated CHS showed antimicrobial property but at different strength of the antimicrobial agent an indication that the strength of antimicrobial activity of the modified CHS depends on the host microorganisms (Gram-positive bacteria and Gram-negative bacteria), molecular weight, viscosity and the degree of acetylation of CHS as this was obvious in the mode of action of the tested microorganisms towards the prepared antimicrobial agent studied in this research work. Therefore, with further investigation and clinical trial it can be used as potent antimicrobial agents for the treatment of infections caused by bacteria.

## ACKNOWLEDGEMENT

We are grateful to the Management of The Federal Polytechnic Auchi and TETfund for making grant available for smooth execution of this research.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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