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Antimicrobial Activity of Biopolymer Extract against Four Selected Microorganisms

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Abstract: The discovery of antibacterial drugs to combat the spread of infectious diseases brought a great relief to the health community which allowed the medical professionals to treat and reduce death rate arising from infections caused by microorganisms. The need to search and formulate more potent and efficient antimicrobial drugs become necessary due to resistance of some microorganisms to available and existing antimicrobial drugs. Chitosan was extracted from crab shell waste through the stages of demineralization, deproteinization, and deacetylation. The chitosan (CHS) was further modified with silver nitrate ($AgNO_3$) solution using various concentrations (0.5, 1.0, and 1.5 M) in order to enhance its antimicrobial property. The crab shell powder (CSP) and chitosan (CHS) were characterized using X-ray diffraction (XRD), Fourier Transform Infrared (FT-IR), Scanning Electron Microscopy coupled with Energy Dispersive Spectroscopy. (SEM-EDS). The antimicrobial activity (zone of inhibition) was carried out using disk diffusion method. The result showed that *E.coli* and *Pseudopodium* showed the highest susceptibility of 10.2 ± 0.2 mm with extract from chitosan treated with 1.5 M silver nitrate (1.5 SNCHE) at 1000 µg/L whereas the least susceptibility was observed to be E. coli (2.0 ± 0.1 mm) with extract from chitosan treated with 1.0 M silver nitrate (1.0 SNCHE) at 250 µg/L. However, untreated chitosan extract (UCHSE) did not exhibit any antimicrobial effect against any of the tested microorganisms. The proximate analysis of CHS and CHN showed % crude protein to be 12.24 ± 0.01 and 20.54 ± 0.03 respectively. The FT-IR spectra of CHS and CHN showed their characteristic absorption peaks and the diffractograms of CSP and CHS revealed $CaCO_3$ to be the major mineral component in the samples.

Keywords: Biopolymer, Microorganisms, Zone of Inhibition (ZI), Extraction, Crab Shell, Silver Nitrate

1. Introduction

Over the years, crab shell wastes have contributed to environmental degradation in most riverine areas and it has been one of the major land pollutants. In order to reclaim good quality of land, the concept of reduce, reuse, and recycle has become a slogan and a way of life most especially in the municipals hence, the need to finding alternatives to its disposal, convert to other useful product and utilizations become inevitable. Materials decomposition or decay is a function of their chemical composition or structure and most products derived from crab shell waste were chitin. Generally, Crabs are decapods crustaceans of the infra-order *Brachyuran* which typically have a very short projecting tail (abdomen) usually hidden entirely under the thorax. They live in fresh water, on land, and are generally enveloped with exoskeleton. Basically the carapace which is the shell on the back of the crab is majorly made of a hard bone called chitin [1] Chitin is a long, naturally abundant, non-toxic and biodegradable, polysaccharide and un-branched molecule consisting entirely of N-acetyl-D Glucosamine units linked by β-1, 4 bonds. It forms the component of the shells of arthropods, lobster, shrimp, crab, octopus, nematodes, fungi, squid, jellyfish, green algae and the exoskeletons of insects [2]. Chitin, after cellulose, is the most abundant polysaccharide in nature [3]. Cellulose is a polymer of D-glucose. Furthermore, chitin occurs in the exoskeleton of arthropods while cellulose occurs in the cell wall of plants and algae. The chemical structure of chitin is similar to cellulose, having one hydroxyl group on each monomer substituted with an acetylamine group. Chitin is a material that gives structural and protective support in the

layer of animals and fungi [4]. Chitosan is derived from chitin when chitin is fully or partially deacetylated. Structurally, it contains β-(1-4 linked D-glucosamine and N-Acetyl-D-glucosamine that is found in the hard outer shells of crustaceans such as crabs, oyster, periwinkle, and shrimps [5], [6]. Chitosan is also biodegradable, biocompatible, bio-adhesive, non-toxic, exhibits antimicrobial property and also use as metal chelating agent (Doxastakis and Kiosseogbu, 2000). The extraction of chitosan begins with the isolation of chitin and treatment of chitin with hot concentration NaOH [7]. The extraction of chitin involves three major stages; demineralization, deproteinization, and decolourization [4].

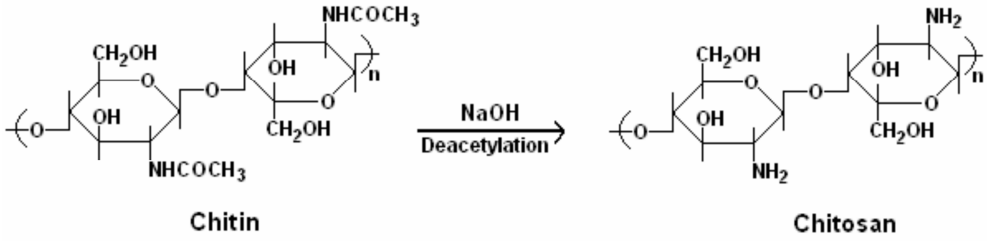

Figure 1. *Chemical Structure of Chitin and chitosan [8].*

Microbes, such as bacteria, fungi and parasites, are regarded as the major cause of infectious diseases, kill more people than other diseases [9]. Among these antimicrobial agents, a lot of attention has been given to chitosan because it has numerous desirable qualities and it is isolated from chitin, which is the second most abundant biopolymer after cellulose in the world [10]. Chitosan exhibits strong antimicrobial effects against various pathogenic and spoilage organisms, [11]. Chitosan have been investigated to possess antimicrobial activities against a wide range of target organisms like algae, bacteria, yeasts and fungi in studies involving *in vivo* and *in vitro* interactions with chitosan in different forms (solutions, films and composites). One of the most studied properties of chitosan is its antimicrobial activity due to the ability of its positively charged amino groups to adhere to the surface of the bacteria wall or the plasma membrane because they have negative charges [12]. The aim and objective of this study is to synthesize chitosan from crab shell waste and investigate its antimicrobial potential

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 dirt 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. Extraction of Chitin and Isolation of Chitosan

2.4.1. Demineralization of the Crab Shell Powder (CSP)

CSP (150 g) was weighed and then 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.

2.4.2. 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 and allowed to stand for 48 hr, 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, stored in a dry container and labeled as CHN

2.4.3. 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.

2.4.4. 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)

2.5. 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

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 0.5, 1.0 and 1.5 M silver nitrate chitosan extract and labeled as 0.5, 1.0, and 1.5 SNCHSE respectively stored and used for the antimicrobial analysis.

2.6. Characterization of Samples

2.6.1. X-ray Diffraction Analysis

The X-ray Diffraction (XRD) is an instrument used for the identification of crystalline phases of inorganic compounds. X-ray diffraction (XRD) analysis of CSP and CHS was carried out to determine 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.

2.6.2. Scanning Electron Microscope with Energy Dispersive X-ray Spectroscopy

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.

2.6.3. Fourier Transform Infrared Spectroscopy Analysis

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 [13].

2.7. Proximate Analysis CHS and CHN

Proximate analysis of CHS and CHN extracted from crab shells were carried out to determine crude protein, crude fibre content, ash content, ether extract, crude fibre, dry matter, ME (kcal/kg) and carbohydrate (CHO) by difference. They were determined by standard method as described by [14].

2.8. Antimicrobial Determination

Peptone water (4.8 g) was weighed into a conical flask and 250 mL distilled water was added and mixed thoroughly. The mixture was heated till it dissolved and the flask was covered and placed in an autoclave for sterilization along with empty test tubes and bijou bottles and was autoclaved for 15 min at 121 ^{o}C. It was allowed to cool and transferred into bijou bottles. Nutrient agar (9 g) was weighed into a conical flask and 250 mL of distilled water was added and mixed thoroughly. The mixture was heated till the solid particles dissolved completely. The flask was covered and the mixture was autoclaved for 30 min at 121 ^{o}C. It was allowed to cool and transferred into Petri dishes. The extracts were tested by the disc diffusion method [15]. The microorganisms; *Styphylococcus aureus, E. coli, Klebsiella pneumonia,* and *Pseudopodium* were inoculated into different bijou bottles and were incubated for 24 hr. The cultured plate seeded with test organisms was allowed to solidify and was punched with a sterile cork borer (70 mm diameter) to make open well. The open well was filled with 50 mL of the extract. The plates were incubated at 37 ^{o}C for 24 hr. The zones of inhibition around each of the antimicrobial agents (the chitosan extracts) disks were measured with a metric ruler. The diameter of the zone of inhibition is related to the susceptibility of the selected test microorganisms and to the rate of diffusion of the antimicrobial agents through the agar medium [16].

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 crab shells powder (CSP) and chitosan (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.

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

Elemental Composition of Crab Shell Powder and Chitosan			
Element	**CSP**	**Weight %**	**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

3.1.1. Scanning Electron Microscopy (SEM) of CHS and CSP

Figure 2 (a) and (b) are the surface morphology of CHS and CPS. The figures revealed the changes in their surface structure. It is obvious 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.

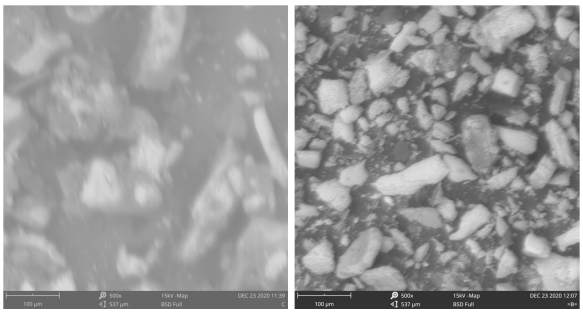

Figure 2. *Showing the Surface Morphology of (a) CHS and (b) CSP.*

3.1.2. 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 crystallinity and mineral composition. The diffractogram obtained from the XRD as shown in figure 3 and 4 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 due to processing stages involved in CHS extraction

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

	Moisture content	% crude protein	% Ash content	%Ether content	% Crude fibre	% Dry matter	ME (kcal/kg) content	% CHO content	Nitrogen content
CHS	7.03±0.04	12.24±0.01	4.1±0.01	48±0.04	3.40±0.01	92.94±0.01	3414.55±1.43	77.80±0.01	1.97±0.01
CHN	6.82±0.04	20.54±0.03	3.80±0.01	6±0.06	3.20±0.01	92.83±0.46	3474.82±1.49	68.83±0.72	3.38±0.12

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

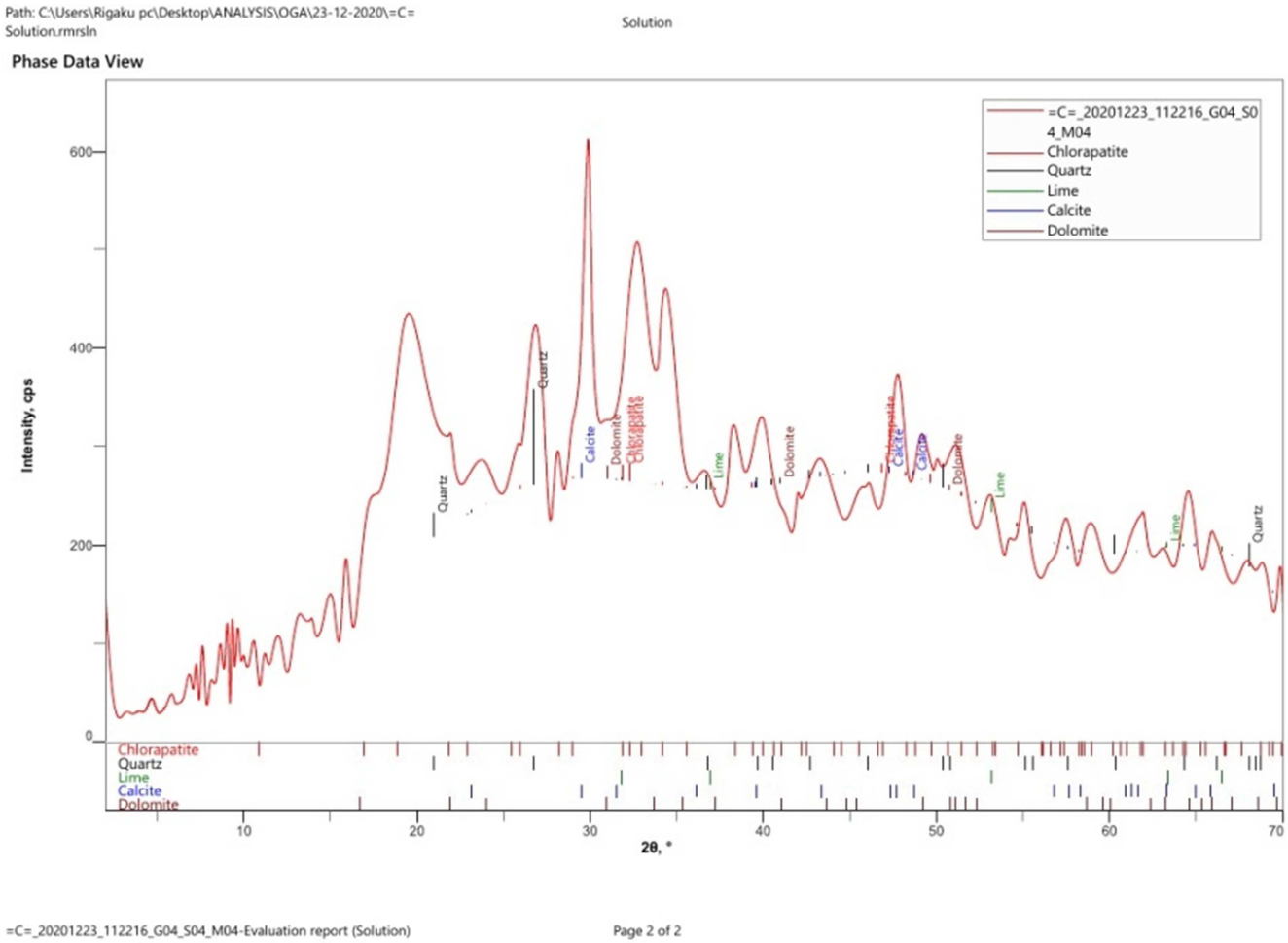

Figure 3. *X-ray Diffraction Diffractogram of CHS.*

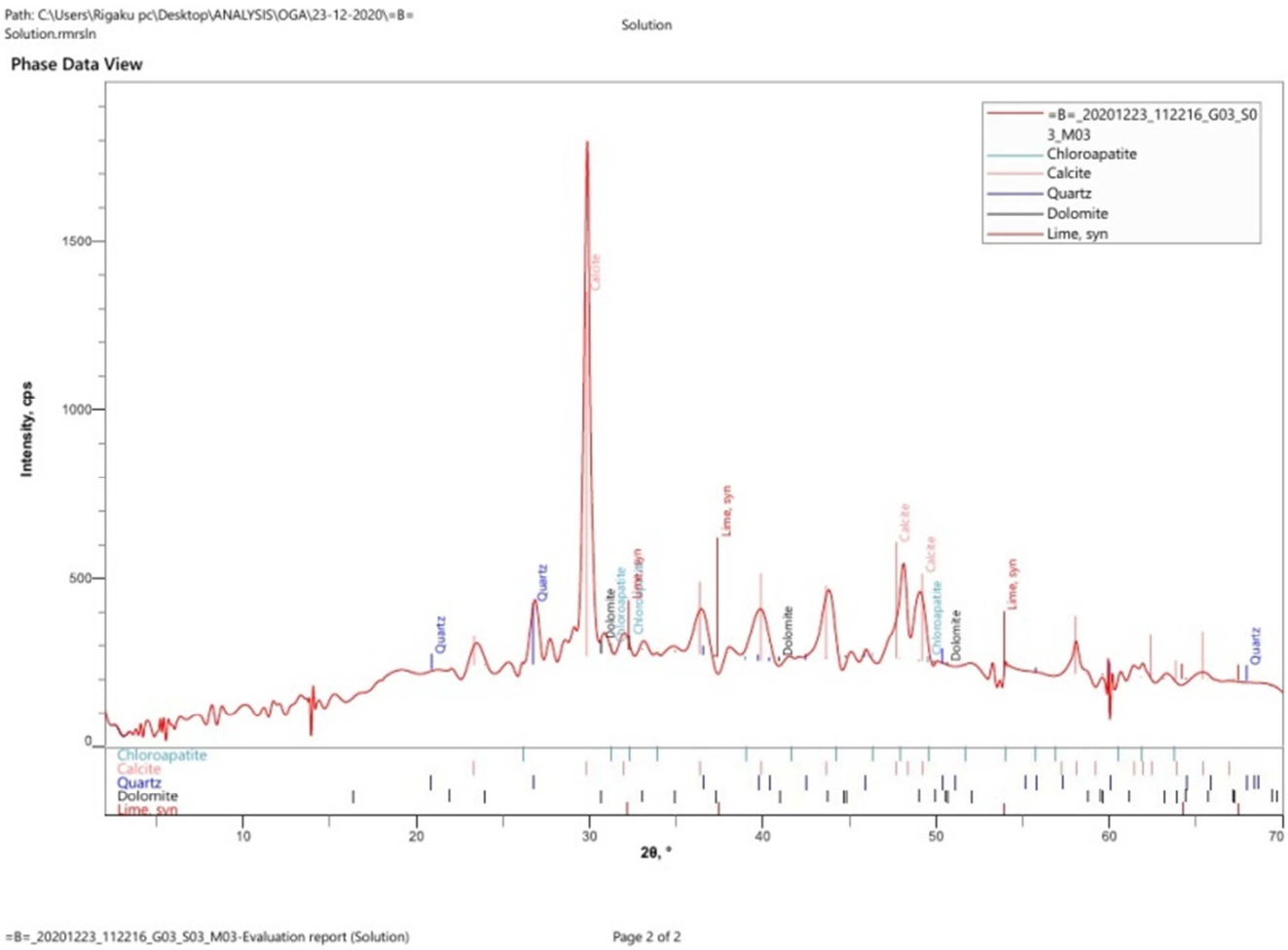

Figure 4. *X-ray Diffraction Diffractogram of CSP.*

3.1.3. 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 [17]. [18] gave a report that moisture content of CHS powder should be lower than 10 % probably due to absorption of moisture from the laboratory environment [19] and from this study it shows that CHS moisture content (7.03±0.04) is of acceptable quality. [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 [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 low concentration of mineral acid (1 M HCl) used during the demineralization stage as evident in higher % CHO content of CHS (77.80±0.01) and CHN (68.83±0.72) indicating high mineral content. The crude protein of CHS was found to be lower which could be as a result of low degree of deacetylation of CHN (CHN contains more nitrogenous substances than CHS). The nitrogen content of CHN (3.38 ± 0.12) was higher than CHS (1.97 ± 0.01). [22] Reported in their work that nitrogen content values greater than 6.89 % indicate the presence of proteins due to low level of deproteinization whereas, nitrogen content values below 6.89 % suggests ineffective demineralization step. The higher fibre content recorded in CHS could be attributed to the removal of more matter from CHN during its deacetylation to yield CHS.

3.1.4. 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). The FT-IR spectra were obtained over a range of 4000 – 350 cm^{-1} using Perkin-Elemer FT-IR spectrum-2 spectrophotometer model. Presented in figure 5 and 6 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 5 is the FT-IR 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 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 $NHCOCH_3$ 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 FT-IR 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 6 is the FT-IR 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.

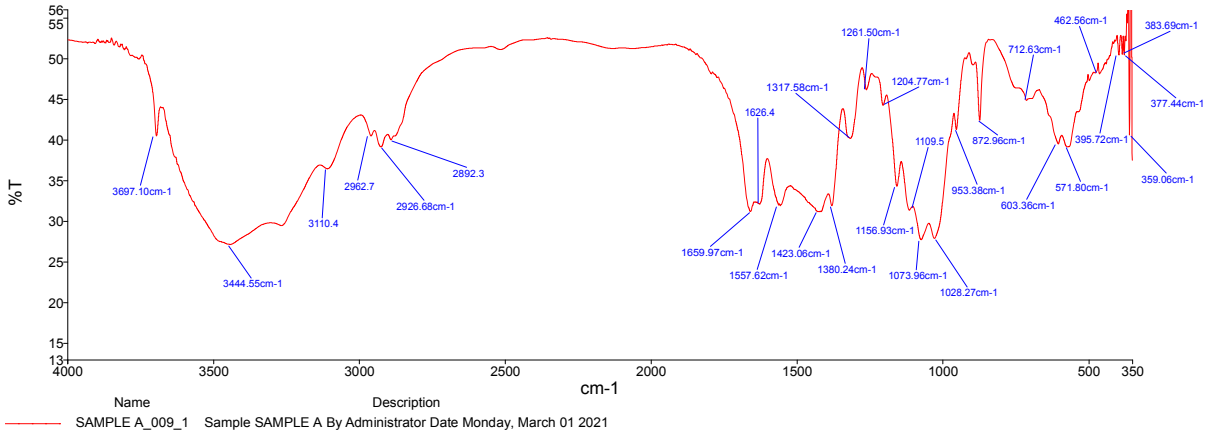

Figure 5. *Fourier Transform Infrared Spectrum of CHS.*

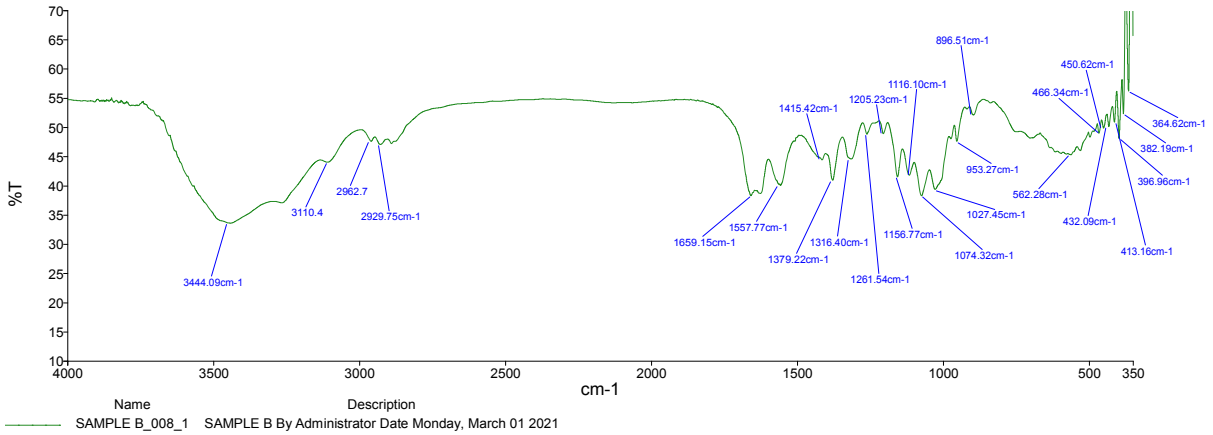

Figure 6. *Fourier Transform Infrared Spectrum of CHN.*

3.2. Antimicrobial Analysis

Table 3. *Zone of Inhibition of the Chitosan Extract.*

Microorganisms	Sample: Antimicrobial Agents/concentration (M)	Sample Dilution (µg/L)/zone of inhibition (mm)				
		1000	500	250	125	62.5
	UCHSE	-	-	-	-	-
E.coli	0.5	4.0±0.1	-	-	-	-
	1.0	8.1±0.1	5.9±0.1	2.0±0.1	-	-
	1.5	10.2±0.2	6.2±0.2	-	-	-
	UCHSE	-	-	-	-	-
Staphylococcus	0.5	7.9±0.1	4.1±0.1	-	-	-
	1.0	10.0±0.1	6.1±0.1	-	-	-
	1.5	10.1±0.1	-	-	-	-
	UCHSE	-	-	-	-	-
Pseudopodium	0.5	4.0±0.0	-	-	-	-
	1.0	3.9±0.1	-	-	-	-
	1.5	10.2±0.2	-	-	-	-
	UCHSE	-	-	-	-	-
K. pneumonia	0.5	-	-	-	-	-
	1.0	-	-	-	-	-
	1.5	6.0±0.1	-	-	-	-

The values presented in the table represent the mean ± SD of two replicates and (-) no inhibition

Table 3 shows the result of the zone of inhibitions (mm) of the extract treated with various concentrations (0.5, 1.0, and 1.5 M) of silver nitrate and the untreated chitosan extracts. The outcome of the study showed that the untreated chitosan extract (UCHE) was unable to inhibit the growth of the selected tested microorganisms at all concentrations. This could be as a result of lower degree of deacetylation of chitin, high molecular weight, and high viscosity. [24] reported that chitosan with high degree of deacetylation possesses a higher positive charge density that increases electrostatic interaction with the negatively charged bacterial cell as a result enhanced microbial potential than chitosan with moderate degree of deacetylation. At 1000 µg/L, *E coli* and *Pseudopodium* showed the highest susceptibility of 10.2±0.2 mm with 1.5 SNCHE. The result form the table also indicate the zone of inhibition of *S. aureus* to be 10.0 ± 0.1 with 1.0 SNCHSE followed by *E. coli* (8.1 ± 0.1 mm) with 1.0 SNCHSE, *S. aureus* (7.9 ± 0.1 mm) with 0.5 SNCHE, *K. pneumonia* (6.0 ± 0.1 mm) with 1.5 SNCHE and the least zone of inhibition (3.9 ± 0.1 mm) was recorded in *Pseudopodium* with 1.0 SNCHE. At concentration of 500 µg/L, the result shows that *E. coli* showed the highest susceptibility of 6.2 ± 0.2 mm with 1.5 SNCHE followed by *staphylococcus* (6.1 ± 0.1 mm) with 1.0 SNCHE, E. coli (5.9 ± 0.1 mm) with 1.0 SNCHE and the lowest was *S. aureus* (4.1 ± 0.1mm) with 0.5 SNCHE. [25] have reported that chitosan antimicrobial potential was a function of dose. The result also reveals that *E. coli* was susceptible to 1.0 SNCHE and has zone of inhibition of 2.0 ± 0.1 mm at 250 µg/L. in this study, the result showed that all the selected microorganisms were resistant to the antimicrobial agents studied at 125 and 62.5 µg/L also *Pseudopodium* and *K. pneumonia* were resistant to the antimicrobial agents at 500 µg/L whereas *K. pneumonia* was only resistant to 0.5 and 1.0SNCHE at 1000 µg/L and *E. coli* was resistant to 0.5 SNCHE at 500 µg/L.

Table 4. *Susceptibility Testing using Standard Antibiotics.*

Microorganisms	OFL	ERY	CXC	AUG	CTR	GEN	CRX	CAZ	NIT
E. Coli	2.95±0.07	-	-	-	-	6.05±0.07	-	-	14.05±0.07
S. aureus	16.1±0.14	4.1±0.14	-	-	-	-	-	-	-
K. pneumonia	-	-	-	-	-	-	-	-	14.1±0.14
Pseudopodium	12.15±0.07	3.95±0.07	-	-	10.05±0.07	6.15±0.07	-	-	-

The values presented in the table represent the mean ± SD of two replicates and (-) no inhibition
Key: OFL (Ofloxacin); ERY (Erythromycin); CXC (Cloxacillin); AUG (Augmentin); CTR (Ceftrazone); GEN (Gentamacin); CRX (Cefuroxime); CAZ (Ceftazidime); NIT (Nitrofurantoin)

Table 4 is the susceptibility test results of some selected standard antibiotics studied. The result shows that *E. coli*, *S. aureus*, and *Pseudopodium* were susceptible to OFL, but *K. pneumonia* was resistant. *E. coli* and *K. pneumonia* were resistant to ERY but *S. aureus* and *Pseudopodium* were susceptible to ERY. The result also shows that only *Pseudopodium* was susceptible to CTR while *E. coli*, *S. aureus* and *K. pneumonia* were resistant. *E. coli* and *Pseudopodium* were susceptible to GEN but *S. aureus* and *K. pneumonia* was resistant to GEN. Also *E. coli* and *K. pneumonia* were susceptible to NIT whereas *S. aureus* and *Pseudopodium* were resistant. All the four tested microorganisms were resistant to CXC, AUG, CRX and CAZ.

4. Conclusion

Chitosan was successfully synthesized from crab shell and modified using different concentrations of $AgNO_3$ to enhance its antimicrobial activity. The results show that UCHSE did not exhibit any antimicrobial effect against all the selected microorganisms which might be as a result of the specie, stage of maturity, source or origin, high molecular weight and viscosity and low degree of deacetylation. The FT-IR spectra of CHS and CHN showed their characteristic absorption peaks and the diffractograms of CSP and CHS revealed $CaCO_3$ as the major mineral component in the samples.

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