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Full Length Research Paper

Production and characterization of derived composite biosorbents from animal bone

Olafadehan O. A.*, Abhulimen K. E., Adeleke A. I., Njoku C. V. and Amoo K. O.

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Composite biosorbents were produced from animal bone by carbonization, activation of animal bone char with phosphoric acid and zinc (II) chloride independently, and the obtained activated carbons were separately impregnated on chitosan. The chitosan was produced from chitin, which was extracted from shrimp shell through demineralization, deproteinization and deacetylation processes. Comprehensive characterization studies were carried out on the chitin, chitosan, and the resulting five biosorbents via proximate and ultimate analyses, and Fourier-Transform Infrared (FTIR), Scanning Electron Microscope (SEM) and Electron-dispersive X-ray Spectroscopy (EDX) analyses. The absorption bands of the standard chitosan from Sigma-Aldrich and the experimentally prepared chitosan were in excellent agreement. The results of this study showed that activated carbons impregnated on chitosan have the potential to be applied as alternative efficient low-cost and eco-friendly biosorbents for batch and continuous adsorption column experimentation.

Key words: Biosorbents, chemical activation, chitin, chitosan, impregnation, characterization.

INTRODUCTION

The most imperative part in the adsorption process is the adsorbent. There is a variety of adsorbents (such as activated carbon, silica gel and alumina) that have been developed for a wide range of industrial applications because they present enormous surface areas per unit weight. However, the significance and relevance of activated carbon to an ever growing society cannot be overemphasized considering its wide applications in domestic, commercial and industrial settings (Mendez et al., 2006) in fluid phase adsorption studies. In the food industry, activated carbon is used in decolourization, deodorization and taste removal. It is used to remove

heavy metals and organic contaminants from industrial wastewater. Activated carbon is used in water dechlorination and processing of foods. It is also used in medicine for adsorption of harmful chemicals and drugs. In gas cleaning applications, activated carbon is extensively used in air filters at industrial level as well as in general air conditioning application (Diets, 1990; Elliot et al., 1989; Inamullah et al., 2008; Oyo and Igbokwe, 2001). Owing to the fact that commercial activated carbon is very expensive and the processes of manufacturing and regeneration are not explicating enough (Bhattacharyya and Sharma, 2004), in recent

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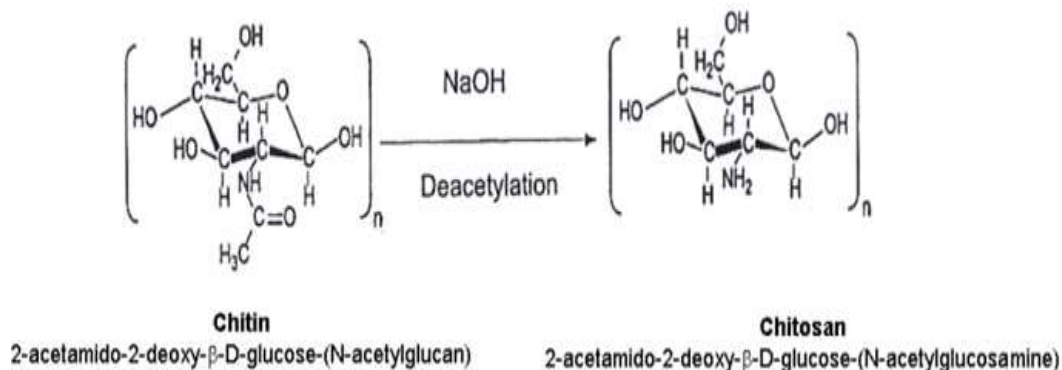


Figure 1. Chemical deacetylation of chitin to chitosan.

years, there is a growing interest in the use of low-cost and abundantly available lignocellulosic materials as precursors for the preparation of activated carbon. Amidst these materials, waste materials from agricultural by-products such as coconut shell, saw dust, bones of animals (cow and goats), and shells of crustaceans are economical and eco-friendly. This is due to their exceptional chemical composition, abundance, renewability, minimal cost, and efficiency in the treatment of industrial wastewater. However, the nature of the final product depends on the physical and chemical properties of the raw material used, the activation procedure (whether physical or chemical) and the process parameters during production (Guo and Lua, 2001; Leimkuehler, 2010). The surface modification of these activated carbons using surfactants may lead to difference in pore size distribution and surface polarity (Douglas et al., 1994), as well as proffering a better remediation for industrial purposes.

Every year, the industrial processing plants of fish and the fish markets do produce enormous crustaceans (shrimp, crab, prawns, lobster and krill) shells as fishery waste that pose environmental hazard. Shrimp shell was utilized in this study to develop added-value products which possess physicochemical and biological properties that find applications in many fields, including adsorption studies. Chitin, 2-acetamido-2-deoxy- β -D-glucose-(N-acetylglucan), is the second most abundant polymer in nature after cellulose. It occurs in nature as ordered crystalline micro fibrils forming structural components in the exoskeleton of anthropoids (Rinaudo, 2006), its major source is from exoskeleton of sea foods (crab, shrimp, prawn, and lobster shells), cartilage of the squid, and outer cover of insects, that are usually disposed as waste material. Depending on its source, three different crystalline polymorphic forms of chitin have been identified: α -chitin (shrimp and crab shells), β -chitin (squid pen), and γ -chitin (stomach cuticles of cephalopoda) (Jang et al., 2004). Chitosan, 2-acetamido-2-deoxy- β -D-glucose-(N-acetylglucosamine), is a biopolymer with free

amine groups. It is produced on an industrial level by chemical deacetylation of chitin with a strong alkaline solution such as NaOH, as shown in Figure 1. Chitosan can also be produced by enzymatic deacetylation of chitin using lysozyme, snailase, neutral protease, and chitin deacetylase (Cai et al., 2006). Chitosan in itself has good adsorptive characteristics but it poses problems for developing commercial applications owing to its slight solubility at low pH, its active binding sites not readily available for sorption and its soft sites tendency to agglomerate or form gel in aqueous solutions. The transport of contaminants to the binding sites plays a very significant role in process design. Therefore, it is imperative to provide physical support and increase the accessibility of the metal binding sites for process applications (Okoya, 2016). However, impregnation of either char obtained from carbonaceous materials or of activated carbon on chitosan (Amuda et al., 2007; Ding et al., 2006; Nomanbhay and Palanisamy, 2005; Wu et al., 2002) has resulted in a diversity of adsorbents with far superior adsorption capacity.

The objective of this study is to contribute in the quest for low-cost and eco-friendly adsorbents that can be used for industrial applications via preparation of char from animal bone (referred to as animal bone carbon, ABC), chemical activation of the char using phosphoric acid and ZnCl_2 , to produce acid-treated animal bone carbon (AABC) and ZnCl_2 -treated animal bone carbon (ZABC) respectively, preparation of chitin from shrimp shell and subsequent preparation of chitosan from chitin, and impregnation of AABC and ZABC on chitosan to produce AABCC and ZABCC respectively, and then provide succinct information on the characterization properties of the five prepared adsorbents. The choice of adsorbents investigated in this study was motivated by the following rationale. Animal (cow) bone, an inexpensive naturally occurring material, is a component of animals. It is available in large quantities as a by-product from vertebrate animals. The shrimp shell from invertebrate crustaceans is a solid fishery waste being disposed thus

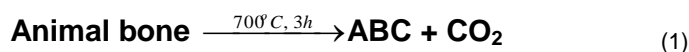
pave way for the preparation of the chitosan in this investigation for subsequent impregnation purposes.

MATERIALS AND METHODS

All the chemicals used were of analytical grade, purchased from Sigma Aldrich in Germany and used as received. These included phosphoric acid and zinc (II) chloride for chemical activation, NaOH (98%) and hydrochloric acid (HCl) (99%) for the preparation of chitosan as well as for pH adjustment, and oxalic acid dihydrate (99.9%) for the preparation of chitosan gel. Also, sodium chloride, iodine solution, sodium thiosulphate and potassium bromide were used at various stages of experimentation. Distilled deionized water was used throughout the experiments for preparing aqueous solutions.

Preparation of char from animal bone

Initially, the animal (cow) bone was thoroughly cleaned using tap water and soap to eradicate possible strange materials present in it (dirt, blood and sands etc). After this, distilled water was used to finally rinse it. The washed bone was sun-dried for 72 h. The carbonization process was carried out as previously described by Amuda and Ibrahim (2006) with a slight modification. The samples were placed in different large crucibles and the set up was kept in a muffle furnace at a temperature of 700°C for 3 h in the absence of air, after which they were removed and kept in a desiccator to allow them cool. Each sample was grounded to a size of 355 µm (44 BSS mesh size). 0.5 M HCl was used to wash and purify the carbon. The product was rinsed several times with distilled water. The sample was then dried in an oven at 100°C for 2 h, and then stored. During this process, the volatile fraction and low molecular products of pyrolysis were removed. The equation for the carbonization process is shown in Equation 1:



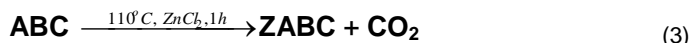
Chemical activation of ABC using phosphoric acid and zinc (II) chloride

The chemical activation of the animal bone carbon (ABC) using phosphoric acid was carried out by the method previously described by Idowu (2015) with a slight modification. 450 g of ABC was charged into a crucible, mixed with 0.8 M of H₃PO₄ and was heated in the oven at 100°C for over 1 h till it formed a paste. The paste in a crucible was taken into a furnace where it was heated at 500°C for 3 h, allowed to cool to ambient temperature and was washed with de-ionized water several times until it reaches pH of 7 (checked by pH meter and litmus paper). It was dried in the oven at 100°C for 2 h. The final product was stored in air-tight polythene bag and put in a dessicator till further use. The equation for the activation process of ABC using phosphoric acid is represented in Equation 2:



The chemical treatment of ABC using zinc (II) chloride was done according to Ningthoujam (2010) with slight modifications. 250 g of ZnCl₂ was added to 1000 ml of water and 250 g of animal bone char was gently added to the mixture and stirred. The mixture was allowed to stay for 24 h; it was drained and dried in the oven at

110°C for 3 h, washed with distilled water to remove traces of ZnCl₂ and dried in the oven 110°C for 1 h. The final product was stored in an air-tight polythene bag till further use. The equation for the activation process of ABC using zinc (II) chloride is shown in Equation 3:



Preparation of chitin from shrimp

The shrimp shell was washed with tap water, and then sun-dried for 4 days. It was later crushed using mortar and pestle into smaller particle sizes and sieved into 1 mm (18BSS). The sieved material was kept in a polyethylene bag at ambient temperature of 28±2°C for 24 h for partial autolysis to facilitate chemical extraction of chitin with a view to improving the quality of chitosan. Demineralization was performed by extracting chitin from shrimp shell as described by Toan (2009) with slight modification. 270 g of the sieved sample was weighed and put in a conical flask. 900 ml of 0.68 M of HCl solution was added (1:5w/v). The mixture was stirred using a griffin shaker at 28°C for 3 h. The resulting solution was filtered and washed till it reaches neutrality (blue litmus paper was used to check for the acidity). It was later scraped into petri dish and dried in the oven at 100°C for 3 h. After this process, deproteinization of the demineralized material was carried out by treating it with 600 ml of 0.60 M of NaOH to form a solution (1:3 w/v). The mixture was stirred and boiled in a water bath at 60°C for 4 h. The resulting solution was filtered and washed with distilled water to neutrality (red litmus paper was used to check if the base had been completely washed off). After washing, the mixture was filtered and the residue (chitin) scraped into a petri dish, and dried in the oven at 100°C for 3 h.

Preparation of chitosan

Deacetylation reaction was used to convert chitin to chitosan according to a revised procedure of Toan (2009). Then, a method described by Aderonke et al. (2014) was used for the preparation of chitosan gel (a whitish viscous gel). The degree of deacetylation of the chitosan was determined by applying the method used by Guibal et al. (1994). The molar mass of chitosan was determined by using the equation of Mark-Houwink-Sakurada for viscosity measurements at different concentrations.

Surface modification of AABC and ZABC with chitosan

The surface modification of AABC and ZABC with chitosan gel was carried out using the methods described by Aderonke et al. (2014) and Babel and Kurniawan (2004) respectively. 150 g of AABC was slowly added to 100 ml of chitosan gel diluted with 500 ml of water, and heated to 50°C and agitated at 150 rpm for 5 h. The resulting AABCC was then soaked in 0.5% NaOH solution till any residual acid was removed. The prepared AABCC was washed with deionized water and dried at 100°C for 2 h, cooled at room temperature and stored in desiccator for use. The same process was applied to 50 g of ZABC to obtain ZnCl₂-treated animal bone carbon impregnated on chitosan (ZABCC).

Characterization of different prepared adsorbents

Determination of surface area

The specific surface area of the activated carbon was estimated

Table 1. Proximate analysis of chitin and chitosan from shrimp shell.

Materials	Proximate analysis, %			
	Moisture content	Ash content	Protein	Fibre
Chitin	23.21	3.63	17.39	46.50
Chitosan	15.40	9.40	14.88	76.40

using Sear method (Alzaydian, 2009) by agitating 2 g of the different adsorbents prepared (ABC, AABC, AABCC, ZABC and ZABCC) separately in 100 ml of diluted hydrochloric acid at a pH of 3. Then, 30 g of NaOH was added while stirring the suspension and the volume was made up to 150 ml with deionized water. The resulting solution was titrated with 0.1 N NaOH to raise the pH from 4 to 9 and the volume, V_{OH} , of NaOH was recorded. The surface area, S , of each adsorbent was calculated using Equation 3:

$$S = 32V_{OH} - 25 \quad (3)$$

The remaining filtrate was cooled to room temperature and the pH was determined using pH meter.

Determination of ash content

The ash content (AC) of each adsorbent was determined by the method of Jeyakumar and Chandrasekaran (2014) by using Equation 4:

$$AC = \frac{M_1}{M \times (100 - X) / 100} \times 100 \quad (4)$$

where M is the mass of sample taken for test, M_1 mass of ash, and X the % of moisture content present in sample taken for test.

Determination of bulk density

The apparent or bulk density, ρ_b , of each adsorbent was determined using the tapping procedure described by Ahmeda et al. (1997). 2 g of each sample of the adsorbent, after being dried at 105°C, was put into a 10 ml capacity graduated cylinder. The bottom of the cylinder was tapped gently on the laboratory bench top several times until there is no further reduction of the sample level. The bulk density was calculated according to the equation: $\rho_b = W/V_M$, where W is the weight of dry material, g; and V_M the volume of dry material, ml.

Determination of pH

The procedure of Jeyakumar and Chandrasekaran (2014) was used to obtain pH. 10 g of the dried sample was weighed and transferred into a 1 L breaker. 300 ml of freshly boiled water (adjusted to pH of 7.0) was added, and heated to boiling. After digesting for 10 min, the solution was then filtered while hot, rejecting the first 20 ml of the filtrate. The remaining filtrate was then cooled to room temperature and the pH was determined using a pH

meter.

Determination of iodine number

The iodine number, I , was determined using the method previously described in ASTM D 4607-94 (2006), and is given by:

$$I = \frac{E \times V_B \times c \times 100}{w_s \times 1000} \quad (5)$$

where E is the equivalent weight of iodine (=127), V_B and c the volume and normality of sodium thiosulphate used respectively and w_s the weight of sample used in g.

Fourier-Transform Infrared (FTIR) analysis

The FTIR analyses of the adsorbents were determined using Shimadzu FTIR-8300 Spectrometer. The corresponding spectra of the chitosan and the derived composite biosorbents were obtained showing the wavelengths in the range 4000 to 400 cm^{-1} of the different functional groups in the samples which were identified by comparison with those in the library.

Scanning electron micrograph of adsorbents

The scanning electron micrograph (SEM) of each adsorbent was carried out using JEOL Scanning Electron Microscope whereby the surface texture and morphological characteristics of the adsorbents were clearly revealed.

RESULTS AND DISCUSSION

Characterization of chitin and chitosan from shrimp shell

Proximate analysis of chitin and chitosan

The proximate analyses of chitin and chitosan prepared from shrimp shell in this study are presented in Table 1. These analyses revealed that the chitin had higher moisture content than the chitosan, which was expected for reason of water being removed from the chitin prior to the chitosan production. The ash content of the chitin was far lower than that of the chitosan as a result of the presence of the acetyl group in the chitin sample. It should be noted that ash is the inorganic residue remaining after water and organic matter had been

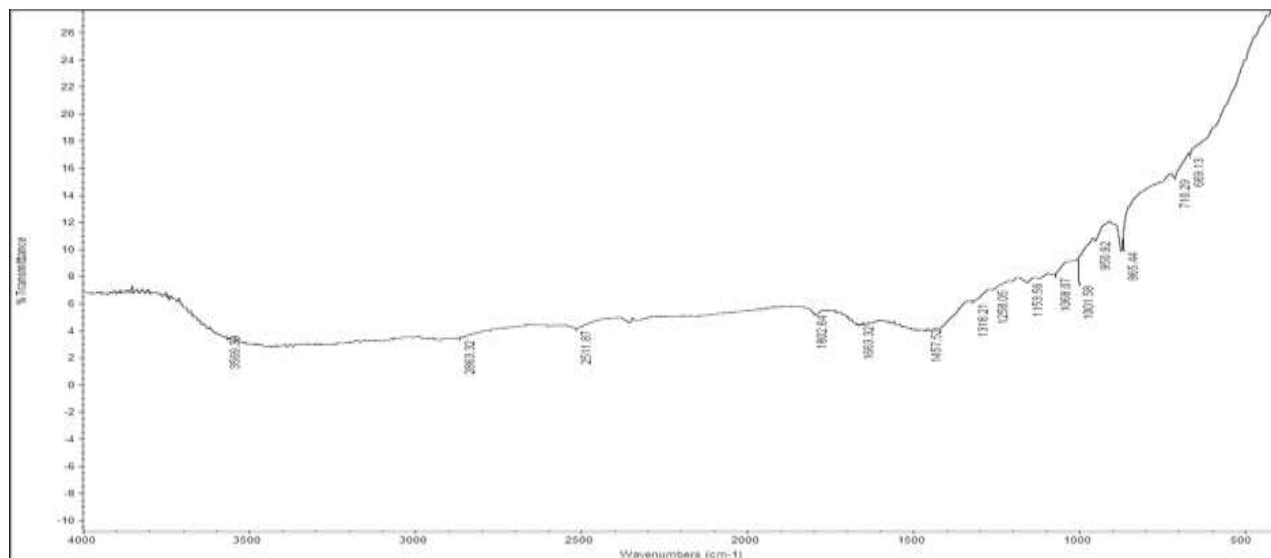


Figure 2. FTIR spectra of the chitin from shrimp shell.

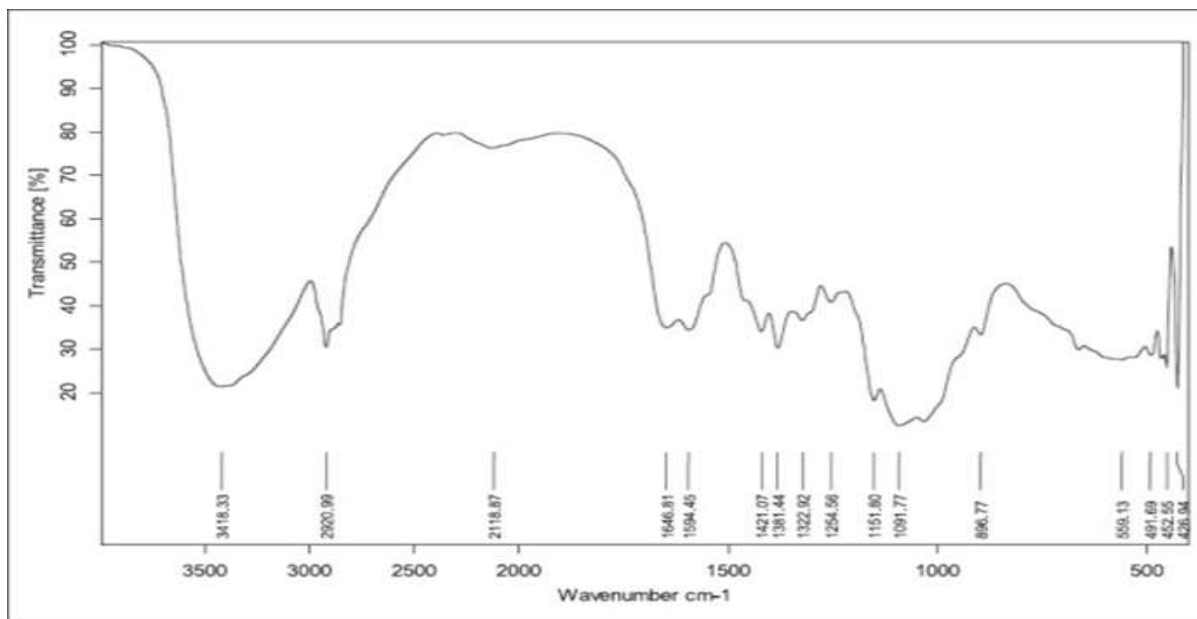


Figure 3. FTIR spectra of chitosan.

removed from a sample. Owing to the deproteinization of the chitin and its low degree of deacetylation of 4.02%, the protein content of the chitosan was less than that of the chitin. The fibre content of the chitosan was higher than that of the chitin owing to the fact that the removal of more matter from the chitin to obtain chitosan could have led to the presence of more fibre in the chitosan than in chitin.

The solubility of the chitosan was checked using four different solvents: water, ethanol, NaOH and concentrated

ethanoic acid. It was found that the chitosan was soluble in acidic condition but insoluble in alkaline, ethanol and neutral solution. The pH value of the chitosan was found to be 10.35.

Fourier Transform Infrared (FTIR) characterization of chitin and chitosan from shrimp shell

Figures 2 and 3 show the FTIR spectra of chitin and

Table 2. Comparison of the characteristic absorption bands in the FTIR spectra of standard and experimentally prepared chitosan.

Wave number (cm ⁻¹)		Functional groups
Chitosan from shrimp shell		
Standard	Experimental	
3423	3418.33	NH ₂ association in primary amines, OH association in pyranose ring
2923 –	2920.99 –	CH ₂ in CH ₂ OH group
2121.8	2118.87	C=O in NHCOCH ₃ group (amide I band)
1667	1648.81	Amide II band (N-H bending)
1597	1594.48	CH ₂ in CH ₂ OH group
1422	1421.07	CH ₃ in NHCOCH ₃ group
1380	1381.44	Amide III band (C-N stretching)
1322	1322.92	Asymmetric bridge oxygen stretching (glycosidic linkage)
1155	1151.80	
1077	1091.77	C-O in secondary OH group
1031	1032	C-O in primary OH group
897	896.77	Pyranose ring stretching
664	663.80	N-H out of plane
616	616	O-H out of plane
Not provided	550.13	C-X group, where X is a halogen

chitosan respectively, where the % absorbance was plotted against wave number. Based on the FTIR spectra depicted for chitin, it was obvious that there was no prominent peak, compared with the one shown for chitosan. Figure 2 was shown to confirm the production of chitosan from chitin, wherein the peaks were assigned to various functional groups according to their respective wave numbers. Various absorption bands within the 4000-400 cm⁻¹ range were recorded in the FTIR spectra of chitosan, prepared from shrimp shell. These bands were compared with those of standard chitosan from Sigma-Aldrich in Table 2. It was evident that there was excellent agreement between the absorption bands of standard chitosan and the experimentally prepared chitosan in this study.

The characteristic band at 3418.33 cm⁻¹ could be assigned to the stretching vibrations of -NH, -OH, -NH₂ and intermolecular hydrogen bonds which overlap each other. The wave number at 2920.99 cm⁻¹ suggested an aromatic ring functional group region. The observed peak at 2118.87 cm⁻¹ was due to the presence of the methyl group in NHCOCH₃. The characteristic -NH band of chitosan indicated the presence of a carbonyl group at the observed band at 1648.81 cm⁻¹ aromatic ring finger print region. It should be noted that the carbonyl group was due to the incomplete deacetylation of chitin to chitosan. The observed peak at 1594.48 cm⁻¹ suggested Amide II band (N-H bending) (Argun and Dursun, 2006). The observed peak at 1421.07 cm⁻¹ suggested aromatic ring finger print region. The wave numbers 1381.44 and 1322.92 cm⁻¹ suggested aromatic rings in the finger print region. The observed band at 1151.80 cm⁻¹ suggested asymmetric bridge oxygen stretching (glycosidic linkage).

The observed peaks at 1091.77 and 1032 cm⁻¹ suggested C-O in secondary and primary OH group respectively. The observed peak at 896.77 cm⁻¹ suggested pyranose skeletal vibrations. The bands at 663.80 and 616 cm⁻¹ were assigned to N-H and O-H out of plane respectively, while the observed band at 550.13 cm⁻¹ was assigned to C-X group, where X is a halogen.

Determination of degree of deacetylation of chitosan

IR technique was used for the determination of the degree of deacetylation, *DD*, of chitosan, according to the methods described by Domszy and Roberts (1985) and Hiral et al. (1991) using Equation 6:

$$DD=100\left(1-\frac{A_{159445}}{A_{292099}}\times\frac{1}{1.33}\right) \quad (6)$$

where A_{159445} and A_{292099} are values of absorbance at the wavelengths 1594.45 and 2920.99 cm⁻¹ respectively, and the factor 1.33 denotes the value of the ratio of A_{159445} to A_{292099} for fully N-acetylated chitosan. From the FTIR analysis of chitosan, the % transmittances at the wavelengths 1594.45 cm⁻¹ and 2920.99 cm⁻¹ were obtained, which was then converted to absorbance (=2-log (% transmittance)) to determine the degree of deacetylation of the prepared chitosan from shrimp shells as 95.98%, using Equation 6. This value was in the range (56-99%) given by No and Meyers (1995), No et al. (2000)

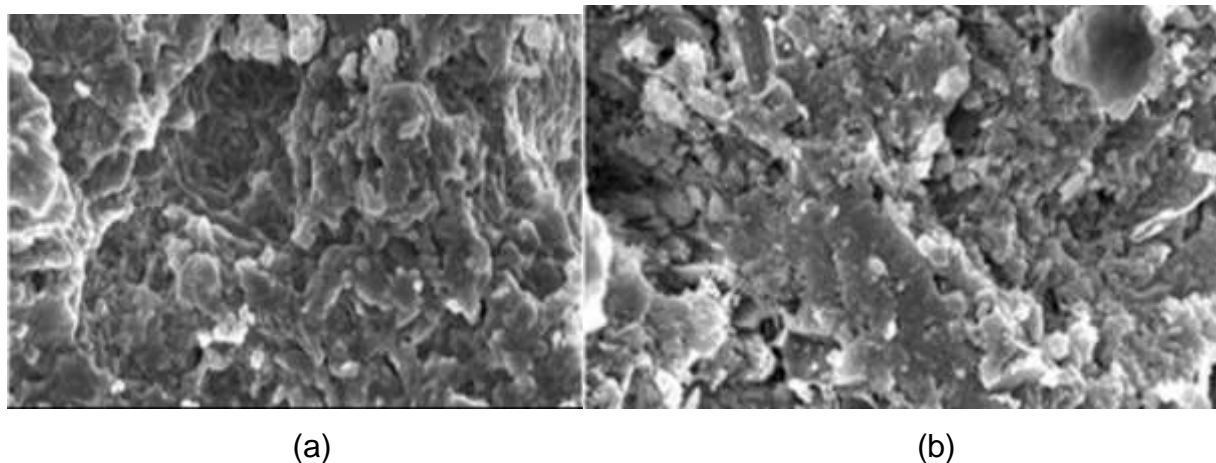


Figure 4. SEM image of chitosan at (a) magnification of 10000; (b) at magnification of 12000.

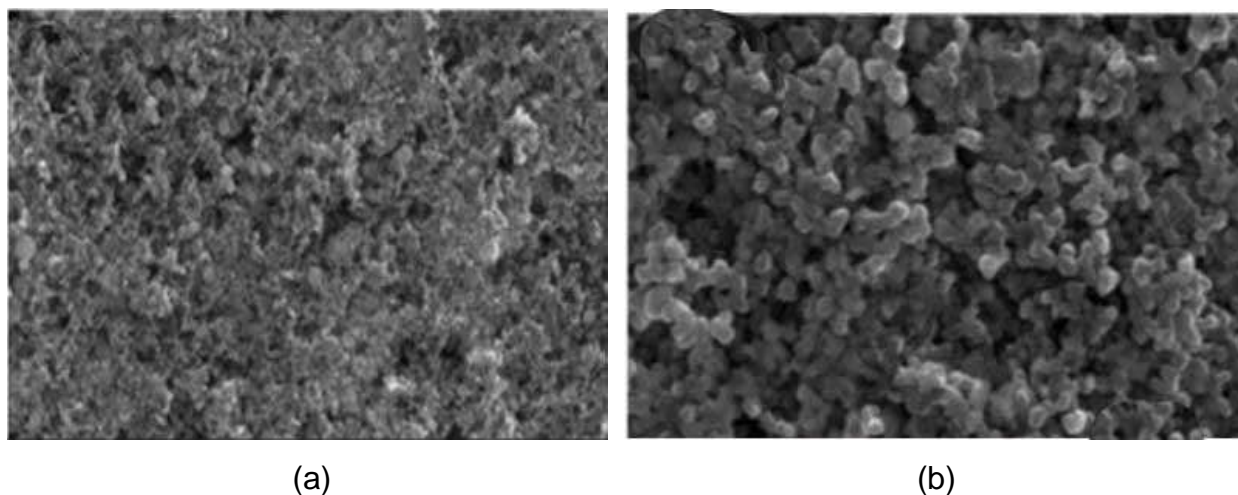


Figure 5. SEM image of animal bone carbon at (a) magnification of 10000; (b) at magnification of 12000.

and Martino et al. (2005), with an average of 80%, depending on the crustacean species and the preparation methods.

Surface morphology of chitosan

JEOL Scanning Electron Microscope (SEM) (model: JSM 7600F) was used to observe the pore structure of the prepared chitosan and animal bone carbon. Figure 4a and b showed the scanning electron microscope images of chitosan produced from shrimp shells at 10000 and 12000 magnifications respectively. The SEM images of animal bone carbon produced from animal bone at 10000 and 12000 magnifications respectively were depicted in Figure 5a and b.

In Figures 4 and 5, the SEM photographs showed a clustered surface with wide varieties of pores present in the prepared chitosan and animal bone carbon, which were more visible at 12000 magnifications with respective average diameters of 5 μm and 2 μm for the chitosan and animal bone carbon. The Energy Dispersive X-ray spectroscopy (EDX) of the prepared chitosan revealed the presence of elements such as carbon (14.20%), silicon (12.30%), oxygen (10.72%), copper (32.10%), zinc (25.35%) and iron (6.55%) in the prepared chitosan, as shown in Figure 6. Similar results were obtained for the animal bone carbon and the derived composite biosorbents with differences in the percentage of elements present being less than ± 0.05 (Figures not shown). The presence of elements such as Zn, Cu, and Fe enhances adsorption of heavy metals through ion

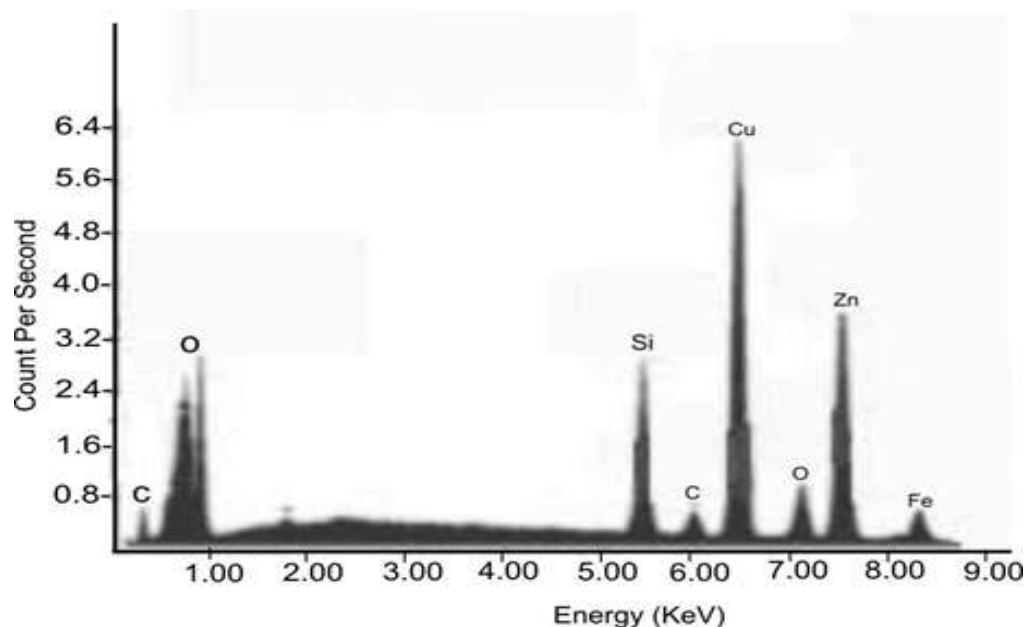


Figure 6. EDX spectra of chitosan.

Table 3. Ultimate and proximate analyses of animal bone.

Ultimate analysis (%)					Proximate analysis (%)			
C	H	N	S	O	Moisture content	Ash content	Volatile matter	Fixed carbon
49.70	1.72	0.20	0.10	48.30	5.50	0.40	75.40	1.50

exchange while elements such as O and Si prompt chelation, coordination and complexation reactions.

Ultimate and proximate analyses of animal bone

The results of the ultimate and proximate analyses of the animal bone are given in Table 3. From the ultimate analysis, the precursor for the preparation of different adsorbents investigated in this study had very low nitrogen and very low sulphur content and thus cannot contribute to environmental issues involving oxides of nitrogen and sulphur. Also, the animal bone has a high volatile matter content and low ash content, which is essential for pyrolysis and gasification processes.

The lignocellulosic composition of the animal bone was determined according to the Robertson and Van Soest (1981) method. Here, the analysis showed the presence of carbohydrate polymers (45.90% cellulose and 14.90% hemicellulose), and an aromatic polymer (39.20% lignin). These carbohydrate polymers contain different sugar monomers (six and five carbon sugars) and they are tightly bound to lignin. They influence the microstructural properties of the carbon. Lignin is the least reactive

substance as compared to cellulose and hemicellulose, it contributes to the solid yield of the thermal process, which definitely influences the final porosity development. The lignin content of the animal bone investigated in this study was comparatively high and thus the precursor would be candidates for the production of a more micropore activated carbon. Heterogeneous pore size distribution depends on cellulose content. High hemicellulose content is associated with having a broader particular pore size distribution in relation to cellulose and lignin.

Characterization of the prepared adsorbents

The proximate analysis of a material is the most often used analysis for characterizing it in connection with its utilisation as an adsorbent. Table 4 presents the characteristics of the five adsorbents (ABC, AABC, AABCC, ZABC and ZABCC) prepared in this study.

Higher moisture content reduces the adsorption capacity of carbon by diluting the action of activated carbon. From Table 4, the moisture content of AABCC

Table 4. Proximate analysis and physicochemical properties of the prepared adsorbents.

Proximate analysis	Adsorbents				
	ABC	AABC	AABCC	ZABC	ZABCC
Moisture content (%)	22.50	17.65	14.40	21.20	18.70
Ash content (%)	6.65	6.00	2.14	8.24	8.12
Volatile matter (%)	7.76	7.76	7.54	7.74	7.60
Fixed carbon (%)	62.42	12.20	10.71	13.10	11.50
Physicochemical properties					
Surface area (m ² /g)	300	397.40	413.40	343	370.20
Bulk density (g/ml)	1.008	1.088	1.687	1.1907	1.4712
pH	8.0	8.33	8.57	8.10	8.62
Iodine value (mg/g)	310	360	470	330	430

was 14.40%, which was the least amongst the five carbons prepared, followed by AABC. Hence, the increasing order of the proposed utilisation of these five carbons as adsorbent is ABC<ZABC<AZBCC<AABC<AABCC, implying that AABCC could be adjudged the best adsorbent in this study. During carbonization, ash content is the inorganic, inert, amorphous and unstable portion present in the activated carbon and it arises from basic nature of raw material (Jeyakumar and Chandrasekaran, 2014). Table 4 reveals that the ash content of AABCC was 2.14% which is the least amongst the five prepared carbon. The lower the ash content, the better the starting material for use as adsorbent. Hence, this investigation revealed that AABCC would be a better adsorbent than the other four adsorbents. The obtained value for AABCC was favourable because the ash content serves as interference during the adsorption process (Khan et al., 2009). AABCC has a lower volatile matter than ABC, AABC, ZABC and ZABCC. This could be attributed to the fact that during thermal activation, most of the non-carbon elements such as hydrogen, oxygen, nitrogen and sulphur might have been eliminated as volatile gaseous products by the carbonization process. There is a little difference in terms of fixed carbon content of AABC, AABCC, ZABC and ZABCC, implying that these carbons are viable options for use as adsorbents in adsorption studies.

The surface area of an activated carbon is directly related to its porosity, and in part to its activity as an adsorbent. From Table 4, the increasing order of surface areas of the prepared adsorbents is ABC<ZABC<ZABCC<AABC<AABCC. Hence, AABCC could be adjudged the best and highly porous adsorbent in this study. However, the surface areas of these adsorbents conformed to the range for plant adsorbents, which is between 10² and 10³ m²/g. The experimental results of bulk density of the prepared adsorbents revealed that AABCC had the highest bulk density, which is indicative of higher quality adsorbent than the other four adsorbents. Thus, AABCC would thus provide greater volume activity thereby resulting in better contact with the

adsorbate, leading to effective adsorption process. The pH parameter is a factor affecting adsorption capacity of adsorbate. It is well known that pH could affect the protonation of functional group on plantain peel as well as metal chemistry (Tsezos and Bell, 1989). The pH values of ABC, AABC, AABCC, ZABC and ZABCC were determined to be 8.0, 8.33, 8.57, 8.10 and 8.62 respectively, as shown in Table 4. These values were in agreement with the findings of Cheremisnoff and Ellerbusch (1978) that the pH of either raw or carbonized materials in water suspension can vary between 4 and 12. Iodine value is a fundamental parameter used to characterize the performance of activated carbon. It is a measure of the micropore content of the activated carbon and is obtained by the adsorption of iodine from solution by the activated carbon sample (Jeyakumar and Chandrasekaran, 2014). Higher value of the iodine value indicates higher degree of activation. The iodine values of ABC, AABC, AABCC, ZABC and ZABCC are given in Table 4. The AABCC had the highest iodine value of 440 mg/g, indicating that its pore surface and structure were the best developed in this study. However, the iodine numbers obtained in this study for the different five carbons are in the range for commercial adsorbents: 300 to 1200 mg/g (Jeyakumar and Chandrasekaran, 2014).

Fourier Transform Infrared (FTIR) characterization of prepared adsorbents

Figure 7 depicts the FTIR spectra of animal bone carbon, where the various functional groups present in ABC were revealed in the 4000 to 500 cm⁻¹ wavelength, as presented in Table 5. The broad band positioned at 3559.89 cm⁻¹ revealed the existence of -OH stretching of the hydroxyl group. The 2359.89-2204.75 and 2014.78 cm⁻¹ bands showed the presence of an alkyne group, C≡C. The peaks at 1416.36 cm⁻¹ indicated C=C groups present in carbon. The observed bands at 1099.74 and 1039.58 cm⁻¹ indicated the presence of a phosphate group, or CO stretching of alcohol group or carboxylic

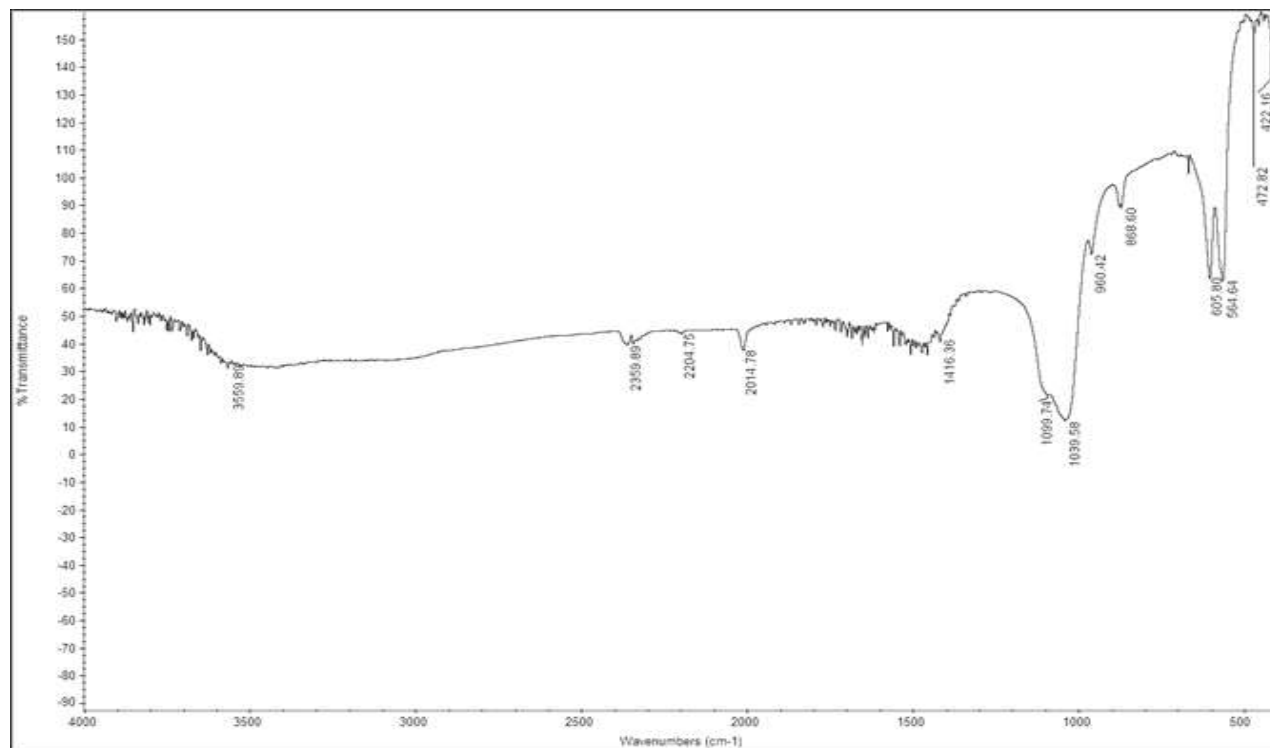


Figure 7. FTIR spectra of animal bone carbon (ABC).

Table 5. Characteristic absorption bands in the FTIR spectra of animal bone carbon.

Wavelength (cm^{-1})	Functional group
3559.89	-OH stretching of the hydroxyl group
2359.89-2014.78	$\text{C}\equiv\text{C}$
1416.36	$\text{C}=\text{C}$, O-H
1099.74-1039.58	PO_4^{3-} , CO stretching for alcohol or carboxylic acid
960.42-868.60	C-H bending of aromatics
605.80-564.64	C-X, where X is a halogen

acid. The peaks around $960.42\text{-}868.60\text{ cm}^{-1}$ showed the appearance of C-H group present in surface of carbon. The peaks between $605.80\text{-}564.64\text{ cm}^{-1}$ indicated the presence of C-X group, where X is a halogen.

Figure 8 shows the FTIR spectra of acid-treated animal bone carbon (AABC) while the different bonds present in it from the FTIR analysis are presented in Table 6. The broad band positioned at 3027.97 cm^{-1} indicated the presence of O-H bond. The bands between 2917.15 and 2207.92 cm^{-1} showed the presence of C-H stretching while the band at 2011.61 cm^{-1} indicated the presence of an alkyne functional group ($\text{C}\equiv\text{C}$). The band at 1419.53 cm^{-1} showed C-F stretching. However, between 1090.24 and 1036.41 cm^{-1} wavelengths, the band width revealed the existence of C=O stretching. At band width of 871.77 cm^{-1} , C-H bending of aromatics was attributed. The

observed band at 602.64 cm^{-1} was as a result of the stretching of halogenated compounds where 'X' represent halogens (chiefly chlorine).

Figure 9 shows the FTIR spectra of acid-treated animal bone carbon impregnated on chitosan (AABCC), where the various functional groups present in it were revealed in the 4000 to 500 cm^{-1} wavelength, as presented in Table 7.

The broad band positioned at 3059.63 cm^{-1} revealed the existence of -OH stretching of the hydroxyl group. The 2014.78 cm^{-1} band showed the presence of an alkyne group, ($\text{C}\equiv\text{C}$). The peaks around 1612.66 cm^{-1} corresponded to the C=O stretching that might be attributed to the lignin aromatic groups. The peak at 1467.02 cm^{-1} indicated C=C groups present in carbon. The observed bands at 1406.86 and 1311.87 cm^{-1}

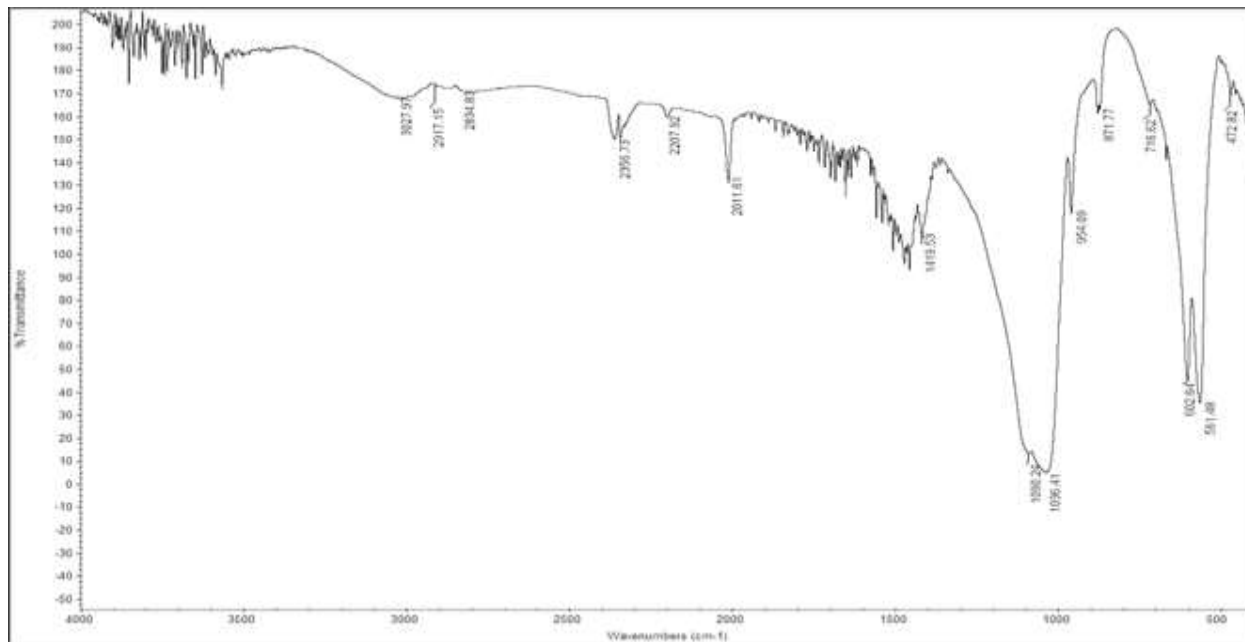


Figure 8. FTIR spectra of acid-treated animal bone carbon (AABC).

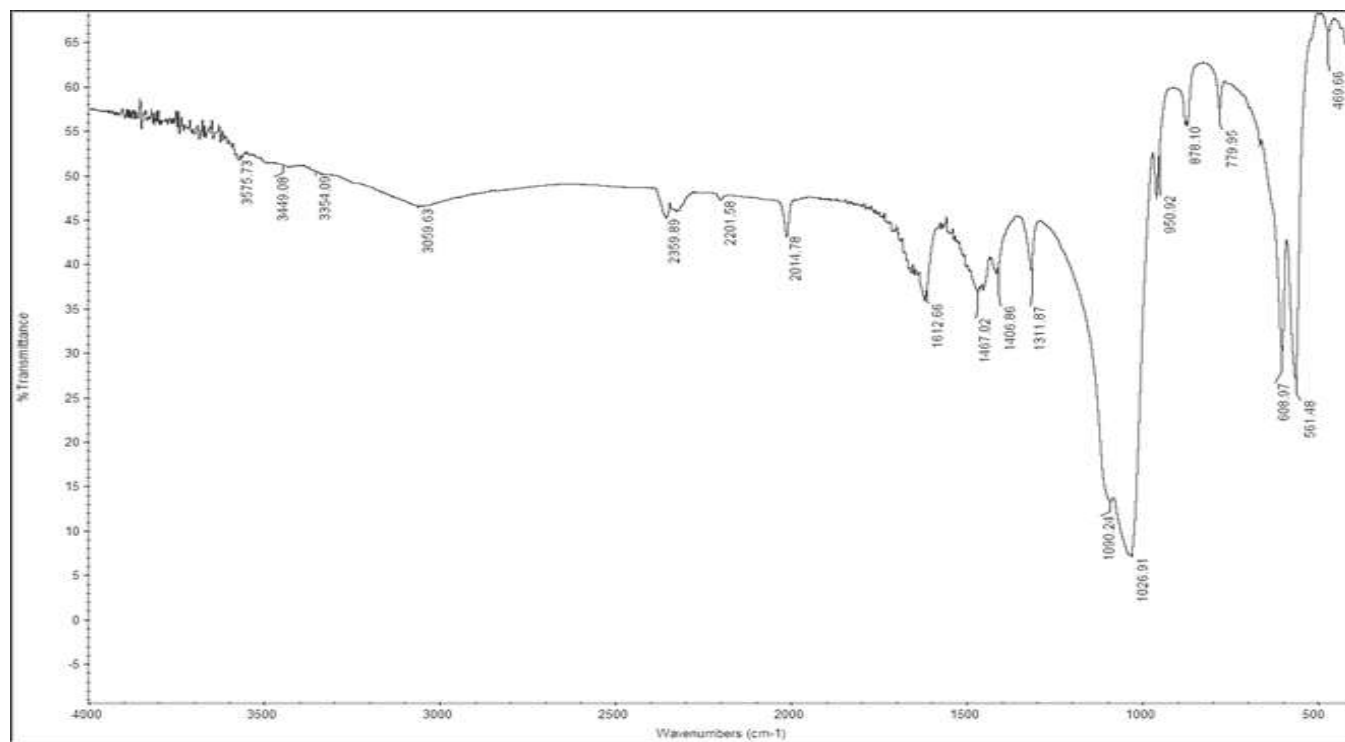


Figure 9. FTIR spectra of acid-treated animal bone carbon impregnated on chitosan (AABCC).

indicated C-F stretching while bands at 1090.24 and 1026.91 cm^{-1} indicated the presence of a phosphate group, or CO stretching of alcohol group or carboxylic

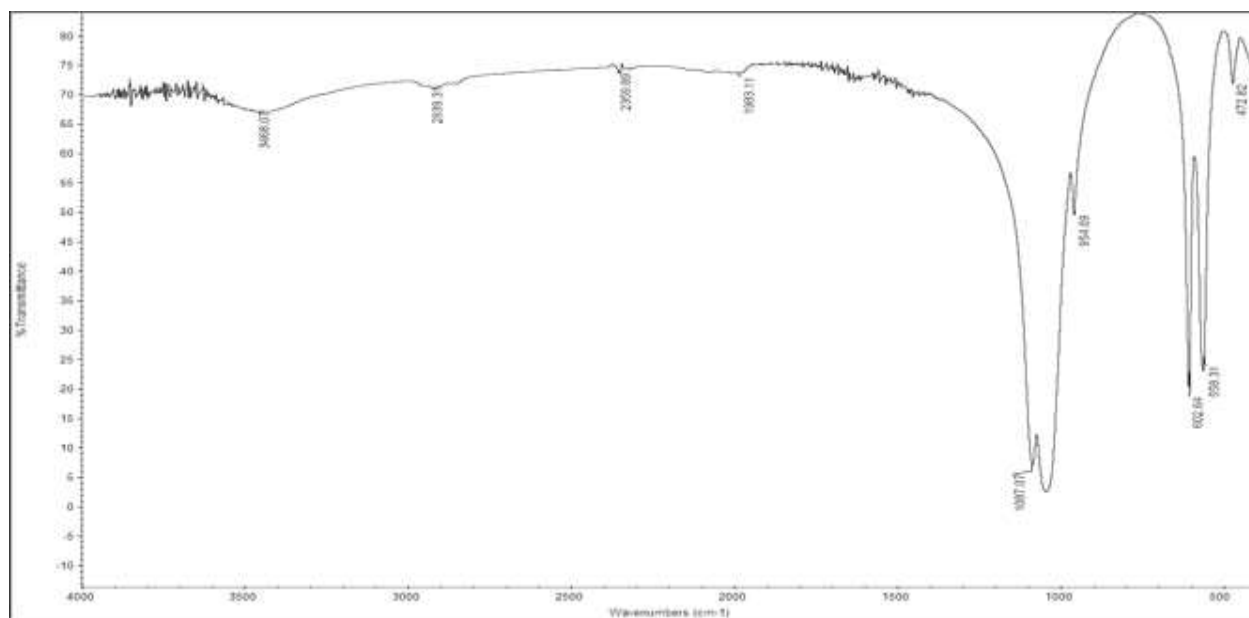
acid. The peaks around 878.1 cm^{-1} showed the appearance of C-H stretching of aromatics. The peaks at 608.97 cm^{-1} indicated the presence of C-X group,

Table 6. Characteristic absorption bands in the FTIR spectra of acid-treated animal bone carbon (AABC).

Wavelength (cm ⁻¹)	Functional group
3027.97	-OH stretching of the hydroxyl group
2917.15-2207.92	C-H stretching
2011.61	C≡C
1419.53	C-F stretching
1090.24-1036.41	C=O stretching
871.77	C-H bending of aromatics
602.64	C-X, where X is a halogen

Table 7. Characteristic absorption bands in the FTIR spectra of acid-treated animal bone carbon impregnated on chitosan (AABCC).

Wavelength (cm ⁻¹)	Functional group
3059.63	-OH stretching of the hydroxyl group
2014.78	C≡C stretching
1612.66	C=O stretching
1467.02	C=C stretching
1406.86-1311.87	C-F stretching
1090.24-1026.91	PO ₄ ³⁻ , C-O stretching for alcohol or carboxylic acid
878.10	C-H bending of aromatics
608.97	C-X, where X is a halogen

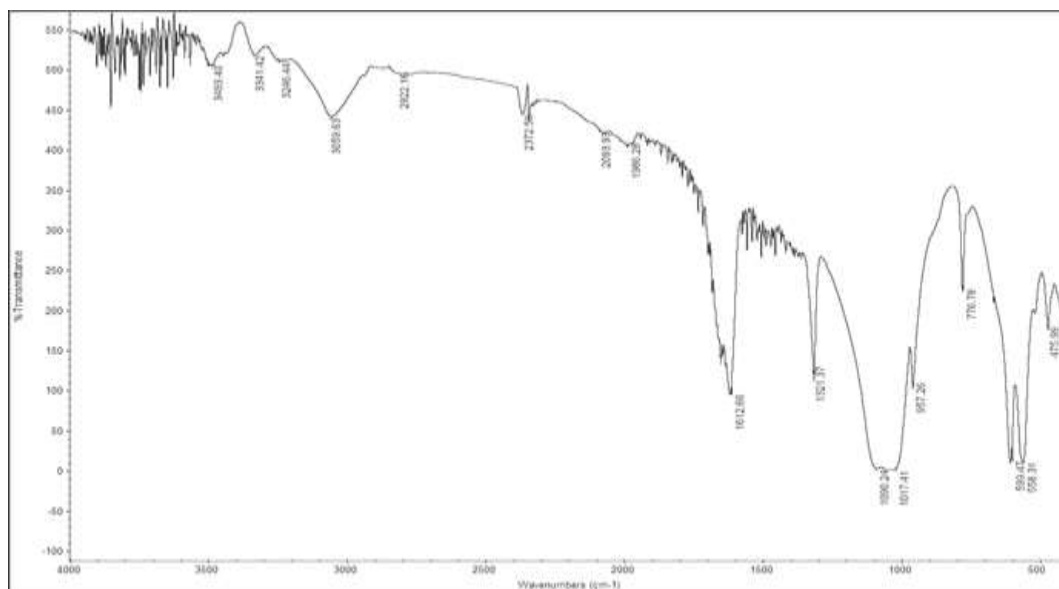
**Figure 10.** FTIR spectra of zinc (II) chloride-treated animal bone carbon (ZABC).

where X is a halogen. Figure 10 shows the FTIR spectra of ZnCl₂-treated animal bone carbon (ZABC) while the different bonds present in it from the FTIR analysis are presented in Table 8.

The 3468.07 cm⁻¹ band showed the presence of O-H stretching of hydroxyl group. The 2939.31- 2359.89 cm⁻¹ band showed the presence of an alkyne group, (C≡C). The bands at 1983.11 cm⁻¹ corresponded to C=O

Table 8. Characteristic absorption bands in the FTIR spectra of zinc (II) chloride-treated animal bone carbon (ZABC).

Wavelength (cm ⁻¹)	Functional group
3468.07	-OH stretching of the hydroxyl group
2939.31-2359.89	C≡C
1983.11	C=O stretching
1087.07	PO ₄ ³⁻ , CO stretch for alcohol or carboxylic acid
954.09	C=H bending of aromatics
602.64-558.31	C-X, where X is a halogen

**Figure 11.** FTIR spectra of zinc (II) chloride-treated animal bone carbon impregnated on chitosan (ZABCC).**Table 9.** Characteristic absorption bands in the FTIR spectra of zinc (II) chloride-treated animal bone carbon impregnated on chitosan (ZABCC).

Wavelength (cm ⁻¹)	Functional group
3493.40-3059.63	-OH stretching of the hydroxyl group
2822.16-2093.93	C≡C stretching
1986.28-1612.66	C=O, O-H stretching
1321.27	C=C stretching
1090.24-1017.41	PO ₄ ³⁻ , C-O stretching for alcohol or carboxylic acid
957.26-776.78	C=H bending of aromatics
599.47-558.31	C-X, where X is a halogen

stretching that might be attributed to the lignin aromatic groups. The observed band 1087.07 cm⁻¹ indicated the presence of a phosphate group, or CO stretching of alcohols and carboxylic acid. The peaks around 954.09 cm⁻¹ showed the appearance of C=H group present in surface of carbon. The peaks between 602.64-558.31 cm⁻¹ indicated the presence of C-X group, where X is a

halogen.

Figure 11 shows the FTIR spectra of zinc (II) chloride-treated animal bone carbon impregnated on chitosan (ZABCC), where the various functional groups present in it were revealed in the 4000-500 cm⁻¹ wavelength, as presented in Table 9. The broad band positioned at 3493.40-3059.63 cm⁻¹ revealed the existence of -OH

stretching of the hydroxyl group. The 2822.16-2093.93 cm^{-1} band showed the presence of an alkyne group, ($\text{C}\equiv\text{C}$). The peaks around 1986.28-1612.66 cm^{-1} corresponds to the $\text{C}=\text{O}$ and $\text{O}-\text{H}$ stretching that may be attributed to the lignin aromatic groups. The peaks at 1321.37 cm^{-1} indicated $\text{C}=\text{C}$ groups present in carbon. The observed band at 1090.24-1017.41 cm^{-1} indicated the presence of a phosphate group, or CO stretching of alcohol group or carboxylic acid. The peaks around 957.26-776.78 cm^{-1} showed the appearance of $\text{C}=\text{H}$ group present in surface of carbon. The peaks between 599.47 and 558.31 cm^{-1} indicated the presence of $\text{C}-\text{X}$ group, where X is a halogen.

Conclusion

Animal (cow) bone carbon was carbonized to produce animal bone carbon, which was subsequently activated with phosphoric acid and ZnCl_2 independently to produce AABC and ZABC. The prepared activated adsorbents were impregnated on chitosan to obtain AABCC and ZABCC. The results showed that activation of ABC improved its surface area and impregnation of AABC and ZABC on chitosan derived from shrimp shell enhanced further their respective specific surface areas. Comprehensive characterization studies were carried out on the chitin, chitosan, and the resulting five biosorbents via proximate and ultimate analyses, and FTIR, SEM and EDX analyses. The results of this study revealed that the use of animal bone and shrimp shells to produce activated carbons and composite adsorbents potentially provides less costly and eco-friendly adsorbents, processed from renewable resources.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Toxicity studies of African Palmyrah palm (*Borassus Aethiopum*) shoots

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The study examined the effect of feeding albino rats with 25, 50, and 75% Palmyrah Palm (*Borassus aethiopum*) shoots with respect to their body weight, liver and kidney function indices. Standard methods of biochemical analyses were employed using albino rats as the experimental animals. No mortality was observed in the LD₅₀ test throughout the period of 48 h. Rats fed with 25 and 50% *B. aethiopum* shoots showed a gradual increase in the body weight throughout the period of treatment, but those fed with 75% of the shoots experienced a significant ($p > 0.05$) decrease in body weight at the 3rd and 4th week of treatment compared to the control group. The results also showed changes in the serum total protein, albumin, globulin, glucose and bilirubin but were not significantly different ($p > 0.05$) compared to the control group. The serum enzymes activities that is aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were significantly ($p > 0.05$) elevated compared to the control group which suggested toxicity induced by some of the phytochemicals present in the feed. Serum creatinine, urea, uric acid, and electrolytes (Na^+ , K^+) of rats fed with 75% *B. aethiopum* shoots showed significant ($p > 0.05$) changes compared to the control group. The results showed that *B. aethiopum* shoots have a relatively low or no toxicity.

Key words: *Borassus aethiopum*, shoots, albino rats, liver function, kidney function.

INTRODUCTION

Food is no doubt the most basic necessity for one to effectively function in his ecosystem. It is a substance that often composed of carbohydrates, lipids, proteins, vitamins and water, which are eaten or drunk by animals or humans for nutrition (Wasagu et al., 2013). The constituent in food contains important chemical substances known as nutrients. These constituents are

ingested, digested, absorbed and circulated in the blood streams to feed the cells that constitute body building blocks. Consequently, the increase in body resistance to diseases and faster recovery of illnesses is witnessed (Worthington-Roberts, 2008).

Most of the foods consumed by humans are sourced from plants and animals; the former has been grouped

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Figure 1. Palmyrah Palm (*Borassus aethiopum*) tree.



Figure 2. Palmyrah Palm (*Borassus aethiopum*) shoots “Muruchi “

into leafy vegetables, seeds, tubers and fruits (Oyiza, 2005). There are over 30,000 known edible plants, from which only 300 were domesticated accounting for more than 95% of the required human plant food (Oyiza, 2005). The part of plant responsible for bearing of seeds is known as fruit and is considered a healthy food supplement because it composed of an appreciable amount of water, carbohydrates, lipids, proteins, vitamins and minerals such as calcium, magnesium, potassium, sodium, zinc, copper and iron (Umar, 2010).

The plant *Borassus aethiopum* (Figure 1) has been described as a palm tree with huge fan shaped leaves (Ahmed et al., 2010). In Nigeria, the Hausas call it *Giginya*, the Yorubas call it *Agbonolodu*, and the Igbos call it *Ubiri*. The plant is a dioecious plant and can reach up to 20 m high on average and 1 m in diameter (Muller, 1988). The shoots of *B. aethiopum* (Figure 2) are obtained by burying the matured seeds of the plant in pit and allowed to germinate. The young germinating shoot or hypocotyls known as *Muruchi* or *Gazari*, is usually harvested after 7 to 8 weeks of planting (Ahmed et al., 2010).

Palmyrah palm (*B. aethiopum*) shoots “Muruchi” is an important source of food for the rural people in Northern

Nigeria. The people consume it either raw or boiled and claimed that it enhances libido in women and aphrodisiac in men (Akinnyi and Waziri, 2011). The shoots are potential source of starch in Cote d'Ivoire, which is an important raw material in industry. Akinnyi and Waziri (2011) reported high concentration of carbohydrate (83.00%) and crude fiber (3.96%) and low crude protein (6.9%) and fat (1.49%) in the shoot of *B. aethiopum* plant on dry weight basis. Similarly, the shoots contained an appreciable amount of both macro and micro mineral elements of which Magnesium (640 mg/100 g), Calcium (433.30 mg/100 g), Potassium (236.70 mg/100 g), Manganese (12.30 mg/100 g), Zinc (12.74 mg/100 g) and Iron (11.50 mg/100 g) are the predominant elements⁷. The research on the nutritional composition of Palmyrah palm (*B. aethiopum*) shoots “Muruchi” conducted by Umar et al. (2015) is generally in agreement with that reported by Akinnyi and Waziri (Akinnyi and Waziri, 2011).

Despite the nutritional benefits of the plant shoots “Muruchi”, anti-nutritional factors such as phytate, tannins, oxalate, hydrocyanic acid and nitrate, which blocked the bioavailability of some mineral elements, are also present in the shoots as reported by Ahmed et al.

(2010). However, the anti-nutrients to nutrients molar ratio are all below the critical level known to impair the availability of some mineral elements such as calcium, magnesium, iron and zinc (Umar et al., 2015).

The use of Palmyrah palm (*B. aethiopum*) shoots "Muruchi" as food is very common especially in areas where the plant grows. Even though *B. aethiopum* shoots are important sources of nutrients and phytochemicals that play important roles in protection against conditions such as cardiovascular diseases and cancer, they also contain other compounds that may cause hepatic/tubular necrosis. The toxicity studies of *B. aethiopum* shoots in this region of Nigeria is not fully documented and hence very scanty. The research therefore, aimed at evaluating the safety of *B. aethiopum* shoots obtained from Sokoto State, Nigeria, by acute and sub-chronic administrations in rats.

MATERIALS AND METHODS

Sample collection and treatment

The matured shoots of *B. aethiopum* plant were collected from the area of cultivation in Kware Local Government Area, Sokoto State, Nigeria. The sample was collected in black polythene bags and transported to laboratory. Prior to analyses, the sample was authenticated at the Herbarium section, Botany Unit, Usmanu Danfodiyo University, Sokoto, Nigeria. The seedlings were dehulled, washed with distilled water, chopped in pieces, milled and then air dried. The dried sample was then pulverized into powder using pestle and mortar and sieved. The powdered sample was stored in a clean polythene bag until when required for analysis.

Toxicological studies

Animals

Albino rats (males and females) weighing 165 to 250 g were purchased from the Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. The animals were kept at the animal house of the department in a wire mesh cages. They were fed with poultry grower's feed and tap water *ad libitum* for two weeks to acclimatize before starting the experiment. Animal treatment and handling was done according to the ethical guidelines reported by Zimmerman (1983) and in accordance with U.S. guidelines as contained in the National Institute of Health guide for the care and use of laboratory animals (National Academy of Science (NAS), 2011).

Acute toxicity study (determination of LD₅₀)

Powdered *B. aethiopum* shoots was fed to five (5) groups of one rat each (one after the other at a grace observation period of 24 h). Another (control group) was fed with grower's feed. All the groups receive tap water *ad libitum*. Observation for toxic symptoms was made and recorded systematically at 1, 2, 4 and 6 h after administration. Finally, the number of survivors was noted after 48 h. The toxicological effect was assessed on the basis of mortality, which was expressed as LD₅₀ and calculated using the limit test dose, up and down procedure of Organization for Economic and Cultural Development (OECD) (OECD, 2001).

Sub-acute toxicity studies

Twenty four female albino rats weighing between 165 – 250 g were group into six groups (five rats per group). The animals were housed in stainless steel and fed with the following diets:

Group 1: Control (100% poultry growers mash).

Group 2: fed with 75% poultry growers mash + 25% pulverized *B. aethiopum* shoots.

Group 3: fed with 50% poultry growers mash + 50% pulverized *B. aethiopum* shoots.

Group 4: fed with 25% poultry growers mash + 75% pulverized *B. aethiopum* shoots.

The body weights of all the animals before and within 28 days (weekly) of treatment were recorded.

Collection of blood and serum

At the end of the 28th day, feed and water were withdrawn from the animals overnight and weighed. The animals were anaesthetized in a container saturated with chloroform vapor and then slaughtered. Blood samples were collected at slaughter into labelled bottles and were used for biochemical assays.

The blood collected was centrifuged at 2000 rpm for 10 min and serum decanted into clean 5 cm³-sample bottle and kept at -20°C until analysis. The sera was used to analyze serum total protein, albumin, aspartate aminotransferase activity, alanine aminotransferase activity, alkaline phosphatase activity, creatinine, bilirubin, urea, uric acid, glucose, electrolytes; while anti-coagulated blood was used for red blood cells count, white blood cells count, platelets count, hemoglobin, packed cell volume and red blood cell indices.

Biochemical analyses

Liver function indices (Serum total protein, albumin, globulins, enzyme activities, total bilirubin, and direct bilirubin) and kidney function indices (Serum creatinine, urea, uric acid, Na⁺ and K⁺) were determined to follow the methods reported by Saidu (2005).

Statistical analysis

The data obtained was statistically analysed using one-way analysis of variance (ANOVA) with SPSS version 10.0 statistical package and the results reported as mean ± standard deviation of the values. Significant difference between the means was determined using LSD at 5% level.

RESULTS

Acute toxicity study (LD₅₀)

Acute toxicity test of *B. aethiopum* shoots produced no mortality after 48 h of observation. However, the sample did not produce any gross negative behavioural changes such as excitement, restlessness, convulsions or coma in the rat, instead reduced reaction to noise was observed.

Body weight (growth performance)

The growth performance of rats fed with control and *B.*

Table 1. Body weight changes of Rats fed with *Borassus aethiopum* shoots.

Dose (%)	Initial weight	1 st week	2 nd week	3 rd week	4 th week
0.00 (control)	166.82±1.48	167.81±1.03	167.91±1.77	168.16±1.92	168.97±1.06
25.00	193.28±0.93	193.74±0.71	194.06±0.91	195.00±0.67	195.74±0.74
50.00	203.53±3.11	204.02±3.44	204.50±3.46	205.32±2.87	205.73±2.61
75.00	205.21±2.75	206.04±2.60	206.93±2.56	205.66±2.51*	205.29±2.61*

Values are mean ± standard deviation. * = Significantly different from the control (P < 0.05) using one way analysis of variance (6).

Table 2. Liver function indices and serum glucose level of rats fed with *Borassus aethiopum* shoots.

Parameters	0% (Control)	25%	50%	75%
TP (g/dl)	6.50 ± 0.66	5.99 ± 0.50	5.73 ± 0.87	5.64 ± 0.24
ALB (g/dl)	4.10 ± 0.77	3.87 ± 0.21	3.62 ± 0.21	3.54 ± 0.24
GB (g/dl)	2.40 ± 1.23	2.12 ± 1.00	2.11 ± 0.12	2.10 ± 0.34
TB (mg/dl)	0.49 ± 0.10	0.51 ± 0.77	0.58 ± 0.49	0.69 ± 0.46
DB (g/dl)	0.33 ± 0.12	0.37 ± 0.29	0.39 ± 0.45	0.48 ± 0.11
TG (mMol/L)	5.12 ± 0.17	4.56 ± 1.88	3.99 ± 1.45	3.58 ± 1.76
ALP (IU/L)	101.7 ± 2.70	319.2 ± 2.9*	406.6 ± 5.9*	453.6 ± 3.0*
ALT (IU/L)	9.33 ± 3.12	13.67 ± 0.58*	23.77 ± 0.68*	31.0 ± 1.00*
AST (IU/L)	26.33±2.93	35.0±3.46*	39.50±3.04*	59.03±0.21*

Values are mean ± standard deviation. TP=Total protein. ALB=Albumin; TB=Total bilirubin. GB=Globulins; DB=Direct bilirubin; TG=Total glucose; ALP=Alkaline phosphatase; ALT=Alanine aminotransferase; AST=Aspartate aminotransferase. * = Significantly different from the control (P < 0.05) using one way analysis of variance (6).

aethiopum shoots (treatment diets) is presented in Tables 1. The result indicates a gradual increase in the body weight throughout the period of treatments at a dose of 25 and 50% not significantly (p<0.05) different compared with the control group. However, there is a noticeable increase in the body weights of rats treated with 75% of the sample at the 1st and 2nd week of the treatment, but at the 3rd and 4th week of the treatment there was a decrease in the body weights which is significantly different (p<0.05) compared to the control group.

Sub-acute toxicity studies

The liver function test

Table 2 presents the liver function indices in rats fed with *B. aethiopum* shoots. There is general decrease in the serum total protein (TP), albumin (ALB), Globulins (GB), and total glucose (TG) in all the treated groups not significantly (p<0.05) different compared with the control, but serum total bilirubin (TB) and direct bilirubin (DB) increased in all the treated groups not significantly (p<0.05) different compared with the control. In all the treated groups serum alkaline phosphatase (ALP),

aspartate aminotransferase (AST) and alanine aminotransferase (ALT) showed a significant (p<0.05) increase compared with the control group.

The kidney function test

Table 3 gives details of the kidney function indices in rats fed with *B. aethiopum* shoots. Serum creatinine, urea, uric acid and potassium showed an increase not significantly (p<0.05) different compared to the control in rats fed with 25 and 50%. Those fed with 75% increase significantly (p<0.05), compared with the control. On the other hand, there is decrease in serum sodium in rats fed with 25 and 50% of the sample not significantly (p<0.05) different compared with the control group, except rats fed with 75% of the sample showed

DISCUSSION

Acute toxicity (LD₅₀) study

The mean lethal dose (LD₅₀) of the *B. aethiopum* shoots to rats did not produce any mortality and did not produce

Table 3. Kidney Function Indices of Rats fed with *Borassus aethiopum* shoots.

Parameters	0.00 (Control)	25%	50%	75%
Creatinine ($\mu\text{Mol/L}$)	81.42 \pm 0.10	88.50 \pm 0.20	90.06 \pm 0.01	104.96 \pm 0.10*
Urea (mMol/L)	9.96 \pm 0.00	10.71 \pm 1.16	11.34 \pm 1.54	14.11 \pm 0.55*
Uric acid ($\mu\text{Mol/L}$)	201.20 \pm 2.20	205.10 \pm 4.10	212.20 \pm 5.00	220.01 \pm 2.20*
Sodium (ppm)	31.30 \pm 3.12	31.06 \pm 0.11	30.00 \pm 0.10	28.00 \pm 0.10*
Potassium (ppm)	8.32 \pm 2.10	8.71 \pm 0.40	8.82 \pm 1.20	9.80 \pm 2.60*

Values are mean \pm standard deviation. * = Significantly different from the control ($P < 0.05$) using one way analysis of variance (6).

any grossly negative behavioural changes such as excitement, restlessness, convulsions or coma in the rat. Instead, reduced reaction to noise was observed suggesting that, the *B. aethiopum* shoots may have depressant effect on the central nervous system and is relatively 'safe' in or non-toxic to rats compared to some known poisonous plants (Hassan et al., 2006).

Body weight (growth performance)

B. aethiopum shoots met all the nutritional requirements in terms of crude protein and available carbohydrate (Akinniyi and Waziri, 2011; Umar et al., 2015). The reduction in the body weight at high dose could be due to reduced feed and water intake observed from the animals, it could also be due to palatability, loss of appetite and more importantly anti-nutritional factors. For instance the high tannins content in the samples may hinder protein bioavailability (Ademoroti, 1996), the saponins present in the sample may have caused loss of appetite (Hassan et al., 2006).

The liver function test

Liver plays an important role in the body considering its function in detoxification of metabolic processes (Helal et al., 2009). The detoxification process disturbs the integrity of cell membrane, which may damage the liver function. Therefore change in the concentration of total protein, albumin, globulin and enzymes (ALP, AST and ALT) in the serum may indicate the state of the liver and the type of damage (Ashafa et al., 2009).

The result of the liver function is presented in Table 3. Total protein is associated with evaluation of hydration status or possible haemorrhage and is a marker for acute and chronic inflammation (Boonprong et al., 2007). From the result, the concentration of serum total protein shows no significant difference ($p > 0.05$) between the control and the sample treatment and is within the rat normal values of 5.6 – 8.6 g/Dl (The Rat Fan Club, 2010). Albumin is synthesized by the liver and is a major form of protein present in blood and is a marker of liver damage (Obob

et al., 2005). The concentration of serum albumin and globulin show no significant difference ($p > 0.05$) between the control and the sample treatment. The decrease in the concentration of serum total protein, albumin, and globulin, and decrease are indication of tissue injury and reflection of hepatic toxicity (Hassan et al., 2007).

Bilirubin is a major breakdown product of haemoglobin. Haemoglobin is derived from red cells that have outlived their natural life and subsequently have been removed by the spleen (Obob and Akindahunsi, 2005). During splenic degradation of red blood cells, haem is separated out from iron and cell membrane components (Jonston, 1999). Haem is transferred to the liver where it under goes further metabolism in a process called conjugation. Conjugation allows haem to become more water-soluble (Obob et al., 2005). The water solubility of bilirubin allows the bilirubin to be excreted into bile. As the liver becomes stimulated, the total bilirubin may rise (Obob et al., 2005). As presented in the result, there is an increase in the serum total bilirubin and direct bilirubin in rats fed with *B. aethiopum* shoots with no significant difference ($p < 0.05$) between the control and the treated groups. This is a clear indication that the sample may not interfere with or reduce the metabolism of bilirubin in the liver as well as increase in haemolysis (Obob et al., 2005; Jonston, 1999; Anosike et al., 2008).

There are many enzymes such as Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and alkaline phosphatase (ALP) that are found in the serum, which do not originate from the extracellular fluid. During tissue damage, some of these biomolecules find their way into the serum through the disruption of cell membranes (Ashafa et al., 2009). Serum enzyme measurement therefore, provides a valuable tool in toxicity studies. The significant ($p < 0.05$) increase in enzymes activity in all the treated groups at high doses of the *B. aethiopum* shoots compared to control implied cytotoxic effect on the liver, which is an indication that the sample is not completely safe if consumed in high quantity. In this study there is a significant ($p < 0.05$) increase in AST and ALT activities are sign associated with the phytotoxins, and are indicative of liver inflammation (Cheesbrough, 1991). Increase in AST level is also associated with increase catabolism of amino

acids in the liver, perhaps due to dietary cyanide (Aning et al., 1998), which is present in *B. aethiopicum* shoots (Umar et al., 2015).

Alkaline phosphatase (ALP) is a marker enzyme for the plasma membrane and endoplasmic reticulum. It is often used to assess the integrity of plasma membrane (Akanji et al., 1993). It is also related to the function of hepatic cell. Increase in serum level of ALP is due to increased synthesis of enzyme in the presence of increasing biliary pressure (Hassan et al., 2007). Significant elevation of serum alkaline phosphatase is an indication of cholestasis with no effective control of ALP activity towards improvement in the secretory function of the hepatic cell (Van and De Broe, 1994).

Glucose level indicates adequate energy supply to animals. Excess glucose is converted to glycogen by the liver. High level of glucose is an indication of certain liver diseases. The result shows a decrease in the concentration of glucose level with no significant difference ($p > 0.05$) between the control and sample treatment and is within the normal values for rat (The Rat Fan Club, 2010).

The kidney function test

The results of kidney function tests are presented in Table 3. The indices (creatinine, urea, uric acid and electrolytes) are required to assess the normal functioning of different parts of the nephrons and are the significant markers of renal dysfunction by the feed consumed (Hassan et al., 2006).

Creatinine is a chemical waste molecule that is generated from muscle metabolism and is produced from creatine, a molecule of major importance for energy production in muscle (Canani et al., 2005). The kidney filters out most of the creatinine and disposes it in the urine (Harita et al., 2008). At high dose (75%), there is a significant ($p < 0.05$) increase in the serum creatinine level compared with the control group. The value is above the normal range of (70 – 95 $\mu\text{mol/L}$) for rats (Ashafa et al., 2009), which is indicative of possible kidney malfunction or renal failure, perhaps due to constituents detected in the sample such as tannins, cardiac glycosides, saponins and flavonoids (Umar et al., 2015).

Uric acid is a product of purine catabolism (Baille et al., 2007). High concentration of uric acid (hyperuricemia) in blood serum causes gout (Friedman et al., 1998). The result indicate a significant ($p < 0.05$) increase in serum uric acid in rats fed with high dose of the sample compared with the control. The significant change indicates a compromised renal malfunction, which could be due to phyto-compounds present in the sample (Umar et al., 2015).

Electrolytes in the body are of great importance for osmotic balance in the body. Sodium and potassium ions are the major extra cellular and intracellular fluid

regulating acid-base balance (Hassan et al., 2007). The results showed a significant difference ($p > 0.05$) at higher dose between the control and the treated group, which suggest that the sample fed to the animal may have significant effect on the variation of body acid-base balance and hence may course some renal dysfunction (Hassan et al., 2006).

Conclusion

From the present findings, it is clear that no mortality was recorded in LD₅₀ test indicating that *B. aethiopicum* shoots is relatively non-toxic. The rats fed with high dose of *B. aethiopicum* shoots experienced decrease in body weight compared to those fed with normal diet, which was attributed to the presence of anti-nutritional factors and non-palatability of the feed. Some biochemical parameters showed that if consumed in high quantity, *B. aethiopicum* shoots might have effect on liver and kidney indices. The results suggest that, *B. aethiopicum* shoots have a relatively low or no toxicity profile. However, the toxicity mechanism(s) of this plant is still undergoing investigation.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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