International Journal of Current Research and Review
DOI: https://doi.org/10.31782/IJCRR.2023.15603

Research Article

IJCRR
Section: Life Sciences

Comparative Characterisation Study of the Properties of Modified and Unmodified African Locust Beans (*Parkia Bigbosa*) Marketed in Ilisan/ Shagamu Area of Ogun State, Nigeria

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ABSTRACT

Introduction: *Parkia biglobosa* is a wide leguminous plant found in Nigeria and many other African countries. It has been found to contain high calorific value, essential proteins, fatty acids etc.

Aims and Objectives: The aim and objective of this work amongst others is to characterize the modified and the unmodified African locust bean with the view of finding out their nutritional value.

Methodology: Scanning electron microscopy (SEM) was carried out on both the unmodified and modified samples at different magnifications in order to determine their morphologies. Also, Fourier transformed Infrared spectroscopy (FT- IR) was employed to investigate the functional groups and their intensities in the water-based extract of both samples. Finally, X-RD analysis was carried out on the unmodified water-based extract in order to determine the phase names available in the sample.

Results: From the SEM analysis, patches and pores were found in each of the samples, but the patches on the modified sample appeared wider than the ones seen in the modified sample. This could be as a result of the breakdown of proteins by microorganisms in the course of fermentation. The following functional groups were detected in the unmodified water sample with their intensities: O-H, N-H (45.64) CH_2O asymmetric, C-H stretch (85.55), CH_2O symmetric, C-H stretch (91.04), C≡N, C≡C (95.16), C=O (64.14) C-CH asymmetric bend (85.22), SO_2 symmetric stretch sulfate (85.43) and C-N Stretch (85.42). Additionally, the following functional groups with their intensities were recorded for the modified water based extract: O-H, N-H (74.75), C-N (82.30), C≡N, C≡C (96.34), C=O (85.07), CO_2 asymmetric stretch (67.44), CH_3 umbrella (74.78), Alkyl/Aryl (83.72), SO_2 stretch sulfate (81.73) amongst other vital result obtained from X-RD analysis.

Conclusion: More functional groups were prevalent in the modified African locust beans seeds than in the unmodified including their intensities. This is indicative of more nutritional value in the modified sample compared with the unmodified sample.

Key Words: Unmodified, Modified, Parkia Biglobosa, SEM, X-RD, FTIR

INTRODUCTION

African locust bean (*Parkia biglobosa*) is a wild legume known for their inexpensive proteins, high calorific value, vitamins, essential amino acids, fiber and essential fatty acids.[1,2]

The word *Parkia* was named after the Scottish explorer Mungo Park, who drowned in river Niger, Nigeria in January, 1805.[3,4,5] Thirty-one species from this genus were reported in 1995,[6,7] and another four more species were discovered in 2009.[8,9]

The tree is especially valuable for its seeds, which are embedded in a yellow pulp inside a brown pod. Each pod contains up to 25-30 seeds. The fermented seed (modified) which is called Iru in Yoruba, or Dawadawa in Hausa, is a popular condiment known to be rich in protein and vitamins.[10,11] An important attribute of the tree is that most of its leaves remain green throughout the dry season and branches are lopped and used as fodder. It contains high amount of essential nutrients, vitamins, minerals, fatty acids and fibre.[12,13,14] The seed are brown-blackish in color, tasty seasoning which are commonly used as condiment in local soups and as a dietary protein source.[15,16] African locust bean pulp, which constitutes about 60-70% of the pod,[17,18,19] has not attracted much attention in terms of research and utilization. *Parkia biglobosa* tree was

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ISSN: 2231-2196 (Print) **ISSN:** 0975-5241 (Online)

Received: 02.02.2023 **Revised:** 21.02.2023 **Accepted:** 03.03.2023 **Published:** 22.03.2023

listed as one of the plants having real wound healing properties in South western Nigeria.[20,21] The plant leaves, bark, root and flowers are also used in the treatment of many diseases such as hypertension, wounds and malaria.[22,23,24] African locust bean seeds contain some phytochemicals that are of health benefit to the body.[25,26,27]

Considering the numerous benefits of *Parkia biglobosa*, it is of note that much work has not been done or reported on the characterization of the unmodified and modified Parkia biglobosa marketed in order to compare the intensities of the functional groups/phytochemicals present in each of such samples respectively. Some users intuitively prefer the unmodified, while others prefer the modified (fermented). Therefore, this work is meant to evaluate that with respect to *Parkia biglobosa* marketed in Ilishan/Sagamu area of Ogun state, Nigeria. From the results of the analyses, a scientific advisory would be recommended in relation to the brand that has much nutritional value or otherwise.

MATERIAL AND METHODS

SAMPLE COLLECTION/ PREPARATION

The *Parkia biglobosa* used in these investigations was obtained from a local market in Ilishan, Remo Ogun state, Nigeria. Ilishan lies within the latitude of 6.8932°E and longitude of 3.7105°N in the rain forest climatic region of Nigeria. The sample was identified at the department of crop science at the Federal university of Technology Owerri, Nigeria with the voucher specimen number of FUT/CR/02/22 by Dr Ikenna Chukwu. The sample was placed in a clean plastic container during transportation to the laboratory.

The unmodified seeds were first soaked in water and thoroughly rinsed, afterwards boiled in order to enhance the removal of the hull from the cotyledons. The cotyledons obtained were dried in an oven-axiom medical UK thermostat oven (DHE-9101-ISA) at 40°C for three days until constant weight was achieved. Additionally, part of the unmodified sample was modified (through fermentation) using standard method. Subsequently, the cotyledons obtained were oven dried using axiom medical UK thermostat Oven (DHE-9101-ISA) at 40°C for three days until constant weight was achieved. Both samples were then grinded with Panasonic mixer grinder (MX-SC2105) and kept in air tight containers prior to analyses.

SAMPLE EXTRACTION

Warm de-ionized water (40°C) were used to obtain extracts from the modified and unmodified locust beans seeds. 30g of both samples were dissolved in 250ml of de-ionized water respectively and were then filtered using muslin cloth according to standard methods.[28,29,30] The extracts were evaporated to dryness using Eyela rotary evaporator (N-1001) Serial number of 60726920 which was equipped with Eyela digital water bath (SB-1000) with a serial number of 10812351.

REAGENTS, GLASSWARES AND EQUIPMENTS USED

All the reagents used in the course of these investigations were of analytical grade. The glass wares used include beakers, measuring cylinder, reagents bottle etc. Other equipments used are Eyela rotary evaporator (N-1001), Eyela digital water bath (SB-1000), Panasonic mixer grinder (MX-AC2105), Golden Mehler USA electronic balance (20002), Axion medical UK thermostat oven (DHE-9101-ISA), Scanning electron microscope, X-ray diffractometer, Fourier transformed infrared spectrophotometer etc.

SCANNING ELECTRON MICROSCOPY (SEM)

The morphological structures of both the unmodified and modified *Parkia biglobosa* was studied at different magnifications using the scanning electron microscope (SU 9000 model) (FEI-Inspect /oxford instrument – X- Max) which was equipped with an energy dispersive x ray (EDAX) spectrophotometer employed for elemental composition analyses.

The sample was firmly fastened to the SEM stub. This was done to ensure that the sample was properly mounted and immobilized on the stub. The height adjustment ring of the sample holder was turned counter clockwise until the mounting surface was in the highest and the stub pin was inserted into the hole on the mountain surface, using the tweezers. Also, it was ensured that the stub was inserted in such a way that the flat of the stub was seated on the mounting surface. The sample was positioned correctly when it was 2mm below the top surface of the holder. Since each one of the vertical marks on the adjustment ring corresponds to 0.5mm, thus rotating the adjustment ring at 4 marks lowered the sample by 2mm. the best resolution was obtained when the sample was positioned 2mm below the holder surface. The holder surface allows for the optimization of the trade-off between maximum field of view (minimum magnification) and the image resolution (maximum useful magnification. When everything was properly positioned, the sample holder was inserted correctly when the sample led lights up green and the message “please load sample” disappeared from the image screen. The door was closed by sliding it down firmly with little initial force. The door locked automatically when a light up orange came up, then the sample loaded and ready for imaging. The phenom was reactivated and the sample automatically moved to the optical imaging position without sample holder ok button was clicked to confirm. The sample holder was activated and its name displayed when the holder was inserted. Phenom door was closed, the sample was transferred automatically to the optical imaging position.

After that, the optical camera was activated and the image displayed in the main viewing window of the image screen, the part of the sample that was magnified in the main viewing window was displayed in the optical overview window.

FOURIER TRANSFORMED INFRA-RED SPECTROSCOPIC ANALYSIS (FT-IR)

In order to determine the functional groups found in the water-based extracts of both the unmodified and modified samples of the Parkia biglobosa, the FT-IR technique was employed.

The spectra of the samples were determined using ATR-FT-IR spectrometer (Agilent FT-IR-7600 Spectrophotometer) which was equipped with a single bounce diamond crystal and a deuterated triglycerine sulfate detector. The FT-IR spectra of the samples were determined in the range of 600-4000cm^{-1} with a resolution of 4cm^{-1}. Each spectrum was collected from 32 scans in the transmittance mode. Triplicate measurements were made and the mean values recorded for each of the samples analyzed. In order to obtain the FT-IR Spectra, the transmittance values were plotted (y-axis) as a function of the wave number (x-axis).

X-RAY DIFFRACTION ANALYSIS (X-RD)

In order to determine both qualitatively and quantitatively the elements present in the water-based extract of the unmodified Parkia biglobosa, the X-ray diffractometer was used. The sample was finely grinded, homogenized, and the average bulk composition was determined. The powdered sample was then prepared using the sample preparation block and was compressed in the flat sample holder to create a flat, smooth surface that was later mounted on the sample stage in the X-RD cabinet. The sample was analyzed using the reflection transmission spinner stage using the reflection transmission spinner stage using the Theta-Theta settings. Two Theta starting position was 4-degrees and ending at 75-degrees with a two-theta step of 0.026261 at 8.67 seconds per step. Tube current was 40MA and tension was 45VA. A programmable divergent slit was used with a 5mm width mask and Gonio scan was used.

The intensity of diffracted x-rays was continuously recorded as the sample and the detector rotated through their respective angles. A peak in intensity occurred when the mineral lattice planes with the d-spacing appropriate to diffract x-rays at that value of Theta. Although each peak consists of two separate reflections (K∝1 and K∝2), at small values of 2θ, the peak location overlaps with (K∝2) appearing as a hump on the side of (K∝1). Greater separation occurred at higher values of θ. Typically, these combined peaks are treated as one. The 2λ position of the diffraction peak is typically measured as the center of the peak at 80% peak height.

Results are commonly presented as peak positions at 2θ and x-ray counts (intensity) in the form of table or an x-ray count (intensity) in the form of table or an x-ray plot. Intensity (I) is either reported as peak height intensity, that intensity above background, or as intensity, the area under the peak. The relative intensity is recorded as the ratio of the peak intensity to that of the most intense peak (relative intensity = $1/\pi$ X 100.

RESULTS

Table 1: XRD qualitative analysis result of water-based extracts of *Parkia biglobosa*

PHASE NAME	FORMULA	FIGURE OF MERIT	PHASE REG DETAIL	SPACE GROUP	DB CARD NUMBER
Tridymite	SiO2	1.506	Import (PDF-4 Minerals 2021)	194: P63/mmc	00-001-0378
Graphite	C	2850	Import (PDF-4 Minerals 2021)	194: P63/mmc	00-008-0415
Silvialite	Ca4Al6(SiO4)6(SO4)	3.093	Import (PDF-4 Minerals 2021)	87:14/m	00-002-0405
Sepiolite	2MgO.3SiO2.2H2O	2.850	Import (PDF-4 Minerals 2021)		00-002-0035
Muscovite	KAl2(Si3Al) O10(OH, F)2	1.654	Import (PDF-4 Minerals 2021)	15:C12/c1	00-002-0467
Osumilite	K-Na-Ca-Mg-Fe-Al-S	1.801	Import (PDF-4 Minerals 2021)	192: P6/mcc	00-010-0413
Marialite	Na4Al3Si9O24Cl	3.057	Import (PDF-4 Minerals 2021)	87:14/m	00-002-0412

Orthoclase	$Al_2O_3K_2O6SiO_2$	2.208	Import (PDF-4 Minerals 2021)	12:C12/m1	00-002-0534
Sulfur, syn	S	1.781	Import (PDF-4 Minerals 2021)	70: Fddd:2	00-008-0247
Garnet	3(Ca, Fe, Mg) O (Al,Fe...	2.850	Import (PDF-4 Minerals 2021)	230:la-3d	00-002-0981

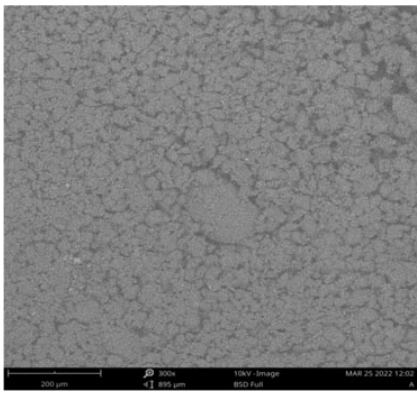

Figure 1: SEM morphology of unmodified *Parkia biglobosa* at (×300) magnification.

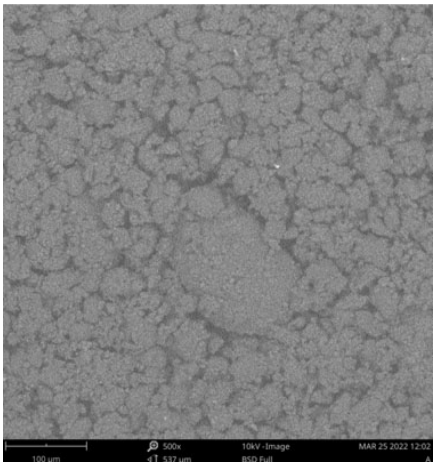

Figure 2: SEM morphology of unmodified Parkia biglobosa at (× 500) magnification.

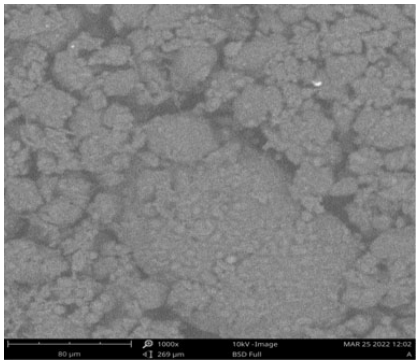

Figure 3: SEM morphology of unmodified Parkia biglobosa at (× 1000) magnification.

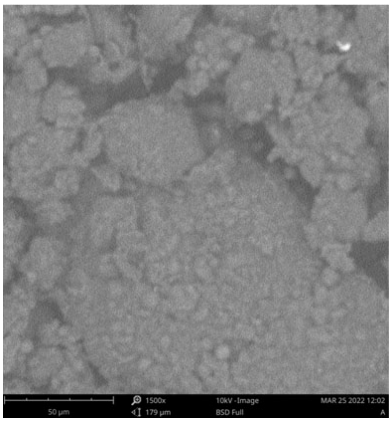

Figure 4: SEM morphology of unmodified *Parkia biglobosa* at (× 1500) magnification.

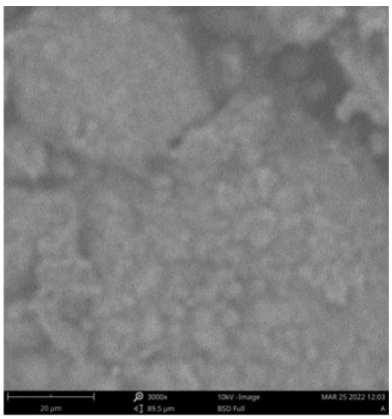

Figure 5: SEM morphology of unmodified *Parkia biglobosa* at (× 3000) magnification.

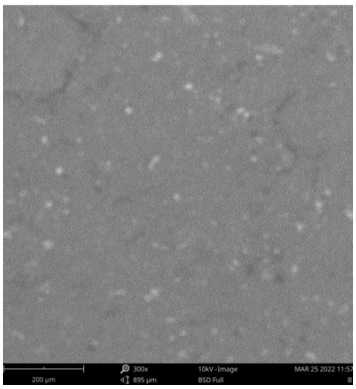

Figure 6: SEM morphology of modified *Parkia biglobosa* at (× 300) magnification.

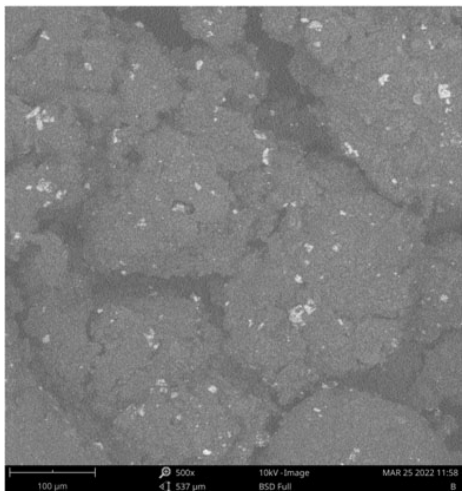

Figure 7: SEM morphology of modified *Parkia biglobosa* at (× 500) magnification.

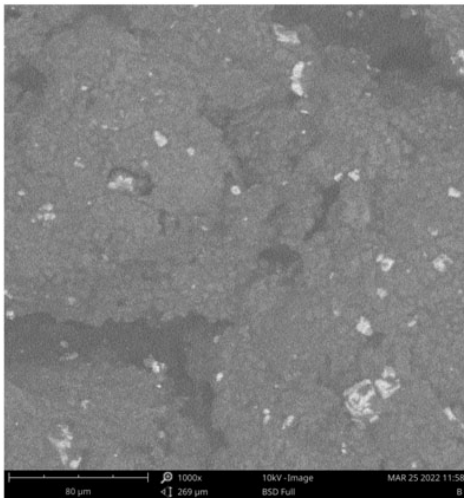

Figure 8: SEM morphology of modified *Parkia biglobosa* at (× 1000) magnification.

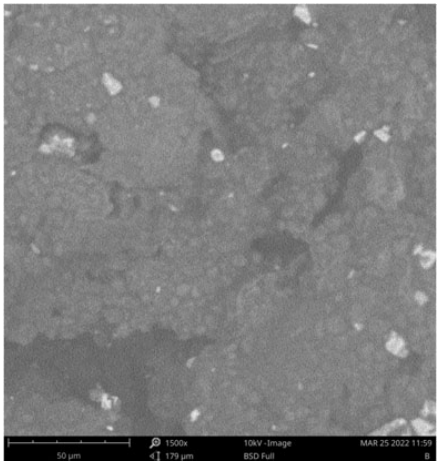

Figure 9: SEM morphology of modified *Parkia biglobosa* at (× 1500) magnification

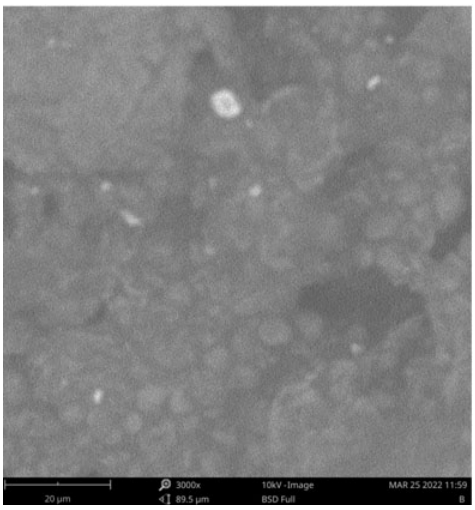

Figure10: SEM morphology of modified *Parkia biglobosa* at (× 3000) magnification.

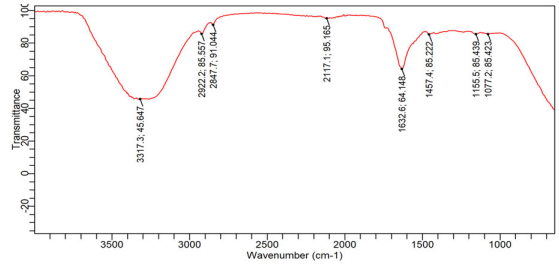

Figure 11: FTIR spectrum of de-ionized water extract of unmodified *Parkia biglobosa.*

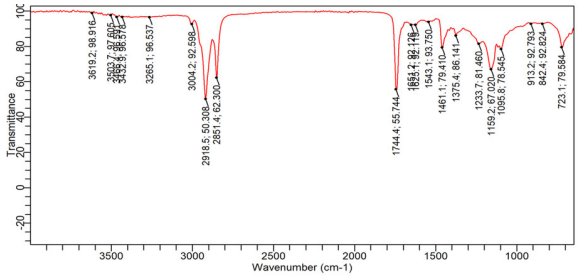

Figure 12: FTIR spectrum of de-ionized water extract of modified *Parkia biglobosa.*

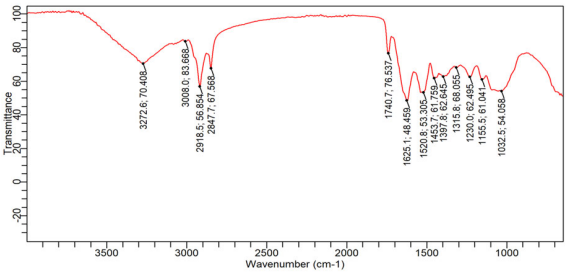

Figure 13: FTIR spectrum of acetone extract of unmodified *Parkia biglobosa*.

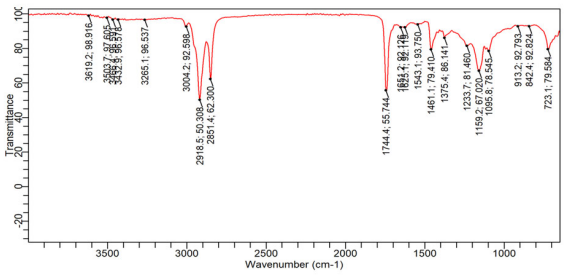

Figure14: FTIR spectrum of acetone extract of modified *Parkia biglobosa*.

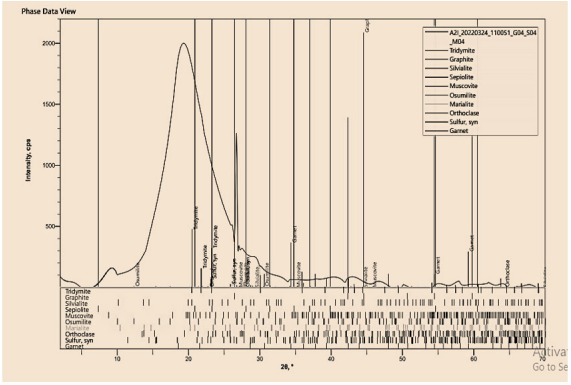

Figure 15: XRD phase data view of water-based extract of *Parkia biglobosa*.

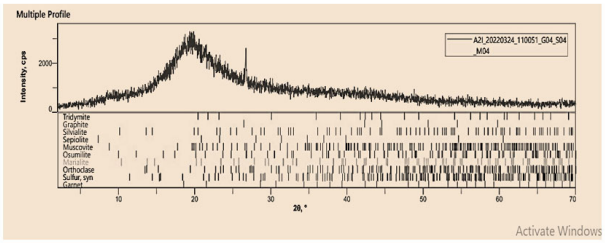

Figure 16: XRD quantitative analysis report of water-based extract of *Parkia biglobosa*.

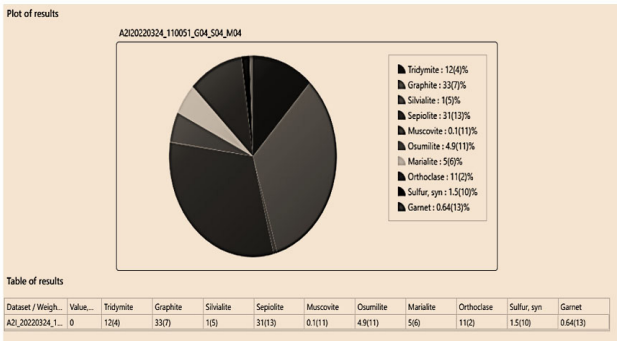

Figure 17: Pie chart plot of XRD of water based extract of *Parkia biglobosa*.

DISCUSSION

Figures (1-5) from the result section show the morphological structure of the modified *Parkia biglobosa* between (X 300-3000) magnifications; while figures (6-10) show the morphological structure of the modified *Parkia biglobosa* at the same magnification levels. As could be seen in figures (1-5), there are evenly distributed patches with some pores in the unmodified SEM diagrams. However, the patches appear enlarged with scattered white patches in the modified sample. The patches are indicative of the fact that some protein molecules have been broken down into smaller molecules in the course of fermentation. A similar report has been reported by other researchers.[15]

Additionally, the white scattered pores seen in figures (6-10) of the modified *Parkia biglobosa* could be attributed to gas diffusion which could be one of the bye products of fermentation. Figures (11 and 12) show the FT-IR Spectra of both the unmodified and modified water-based extracts respectively. Some similar functional groups were found in both the unmodified and modified water-based extracts as shown in the result. However, the intensities of these group vary, with greater proportions found in the modified sample. This is attributed to the breakdown of some protein molecules in the course of the fermentation.[16]

Additionally, some other functional groups were noticed in the modified water sample which were absent in the unmodified sample as shown in the result section. This is indicative of the fact that fermentation actually occurred.

Table 1 shows the X-RD qualitative analysis of the unmodified water-based extract of *Parkia biglobosa*. Ten different phase names were identified in the sample as shown in the result section.

The elemental make ups of these phase names were equally identified. *Seniolite, osumilite* and garnet were found to contain important essential trace elements necessary for human body metabolism as shown in figures 15-17.

CONCLUSION

This work identified similar and different functional groups in the unmodified and modified *Parkia biglobosa* water-based extracts. However, the intensities found in the modified appeared bigger than the ones found in the unmodified sample. This is suggestive of higher nutritional value in the modified sample over the unmodified. Additionally, the X-RD qualitative elemental analyses revealed the presence of several micro elements that are necessary for human body metabolism. These elements include Ca, K, Na, Mg, Fe etc. This implies that the consumption of *Parkia biglobosa* should be encouraged especially for patients who lack these basic essential micro elements.

ACKNOWLEDGEMENT

We acknowledge all who played one role or the other in the course of this work. Specifically, to be mentioned include Dr Ikenna Chukwu of the Department of Crop Science, Federal University of Technology Owerri where the sample was identified and Management and staff of the central research laboratory at the Yaba college of Technology, Lagos where some analyses were carried out.

Source of funding: Personal funding from the authors.

Conflict of Interest: None.

Authors' Contribution: Idika Digbo I, Anaekwe Vera C and Ndukwe Nelly A, the above listed authors contributed equally in the course of this work.

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