COMPARATIVE STUDY OF THE BIOACTIVE COMPOUNDS IN ACETONE EXTRACTS OF MODIFIED AND UNMODIFIED AFRICAN LOCUST BEAN *(PARKIA BIGLOBOSA)* MARKETED IN ILISAN/SHAGAMU AREA OF OGUN STATE, NIGERIA.

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ABSTRACT

Parkia biglobosa is a wild leguminous plant found in Nigeria and many other African countries. It has been found to contain high calorific value, essential proteins, fatty acids, and also could be used in the production of antibacterial recipes. The objective of this study amongst others was to characterize the modified and the unmodified *Parkia biglobosa* with the view of finding the type that could best be used in the formulation of antibacterial, fungicidal and antimicrobial recipes. Scanning electron microscopy (SEM) was carried out on both samples at different magnifications in order to determine their morphologies. Fourier transformed infrared spectroscopy (FT-IR) was employed to investigate the functional groups and their intensities in the acetone extract of both the unmodified and modified sample extracts. 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 on the unmodified 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 acetone sample with their intensities: O-H, N-H (70.47),(3200-3600 cm^{-1}) C-H (67.56),(3000-3100 cm^{-1}) N-H in plane mode (53.30),(3300-3500 cm^{-1}) SO_2 stretch sulfate (61.04), C-N stretch (68.05),(1500-1650 cm^{-1}). For the acetone based modified extract, we have the following: O-H, N-H (96.59) C-N (92.59) C=O (92.11), N-H in plane bend (93.75), C-CH_2 scissors (79.42), C-N stretch (81.46), C=C (92.82), C-CH_3 symmetrical bend (86.14), SO_2 stretch (67.01) and benzene ring (73.82). More functional groups were prevalent in the modified *Parkia biglobosa* than in the unmodified including their intensities. This could be indicative of the enhanced antimicrobial, ant fungicidal activities of the modified sample.

Keywords: modified, FT - IR,acetone, SEM, *Parkia biglobosa*

Introduction

African locust bean (*Parkia biglobosa*) soy bean (*Glycine max*.), Bambara groundnut (*Vigna subterranean*) ogiri is traditionally prepared by fermenting melon seeds (*citrullus vulgaris*) and fluted pumpkin (*Telfairia occidentalis*) or castor oil seed (*Ricinus communis*) Folarin and Oluwajenyo, 2004).

Parkia biglobosa is a dicotyledonous angiosperm belonging to the family *Fabaceae* (*caesalphinoideae-mimosoid clade*). It is categorized under spermatophyte, vascular plant. It is a deciduous perennial plant that grows to between 7 and 20 meters high, in

some cases up to 30 meters. The tree is a fire resistant heliophyte characterized by a thick dark brown bark. The pods of the tree commonly referred to as locust beans, are pink in the beginning and turn dark brown when fully matured. Each pod can contain up to 30 seeds. The seeds are embedded in a sweet, powder classification (Thiombiano *et al*., 2012). Where the tree is grown, the crushing and fermenting of these seeds constitutes an important economic activity. Various parts of the locust bean tree are used for medicinal purposes. As a standing tree, locust bean may have a positive effect on the yield of other nearby crops (Nitui *et al*., 2012). Soaking of *parkia* is primarily done to soften the seed coats. During this process, the seed increases in mass due to water absorption.

According to (Germah *et al*., (2007)), soaking also aids the dispersion of water into the starch granules and protein fraction, which subsequently facilitates starch gelatinization and protein denaturation as well as softening of the legume structure.

Fermentation step is vital and is the required biochemical process that transforms the legume through microbial action. Fermentation is usually carried out to purposely enhance sensory properties including aroma, taste and appearance. It is fermented through an alkaline fermentation, where Bacillus species modifies the substrate through proteolytic modifications resulting in increased alkalinity (Adebo *et al*., 2018)

The sugar content of *Parka biglobosa* is found to be 9.0%. It is also a ready source of energy, since it is more easily digested and absorbed than other complex carbohydrates (Germah *et al*., 2007). Bello *et al*.(2008) reported a high calcium content of 1165mg/kg for *parkia* fruit pulp. The recommended dietary allowance for calcium is 800mg/day. He further stated that *parkia* fruit pulp has a magnesium content of 7000mg/kg. Magnesium plays a major role in relaxing muscles along the airways to the lungs, thus allowing asthma patients to breathe easily.

The phytochemical screening by Abioye *et al*, (2013) revealed the presence of alkanoids, flavonoids, tannins, saponins, steroids, glycoside and cardiac glycosides which are active against bacteria or diseases. The importance of the acetone based extracts cannot be over emphasized due to its antimicrobial, bactericidal and fungicidal activities of the extracts.

Considering the numerous benefits of *Parkia biglobosa*, it is of interest to observe that not much work has been done or reported on the characterization of the unmodified and modified acetone based *Parkia biglobosa* marketed in Ilisan/ Shagamu Area of Ogun State, Nigeria in order to compare the intensities of the functional groups present in each of the sample extracts respectively.

Therefore, this work is meant to evaluate that with respect to *Parkia biglobosa* marketed in Ilisan/ Sagamu area of Ogun state, Nigeria. From the results of the analyses, a scientific advisory would be recommended in relation to the extract that would be more suitable for the production of antimicrobial, bactericidal and fungicidal recipes.

Experimental

Materials and methods

Sample collection and preparations

The *Parkia biglobosa* used in these investigations was obtained from a local market in Ilisan, Remo, Ogun state, Nigeria. Ilisan lies within the latitude of 6.8932°E and 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 number FUT/CR/04/22 by Dr Ikenna Chukwu.

The sample was placed in a clean container during transportation to the laboratory. The unmodified seeds were soaked in water and thoroughly rinsed, afterwards boiled in order to remove 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. Both samples were then grinded with Panasonic mixer grinder (MX – SC 2105) and kept in air tight containers prior to analyses.

Sample extraction

Acetone was used to obtain extracts from the modified and unmodified locust bean seeds. Seed extracts were obtained by maceration as 30g of the samples were dissolved in 250mL of acetone and were left to stay for 24 h. The extracts were filtered using muslin cloth and the aqueous filtrates were evaporated to dryness using (78HW-1) constant temperature magnetic stirrer maintained at 40°C inside a fume cupboard.

Scanning Election Microscopy (SEM)

The morphological structures of both the modified and unmodified *Parkia biglobosa* was studied at different magnifications using the scanning electron microscope (SV 9000 model) (FEI-Inspect/oxford instrument-x-max). The sample was firmly fastened to the SEM stub. The height adjustment ring of the sample holder was turned counter clockwise until the mounting surface was in the highest and 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).

Fourier Transform Infra-Red Spectroscopic Analysis (FT-IR)

In order to determine the functional groups found in the acetone-based extracts of both the modified and unmodified 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 deuterated triglycerine sulfate detector. The FT-IR spectra of the samples were determined in the range of 600-4000 cm^{-1} with a resolution of 4 cm^{-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).

Results

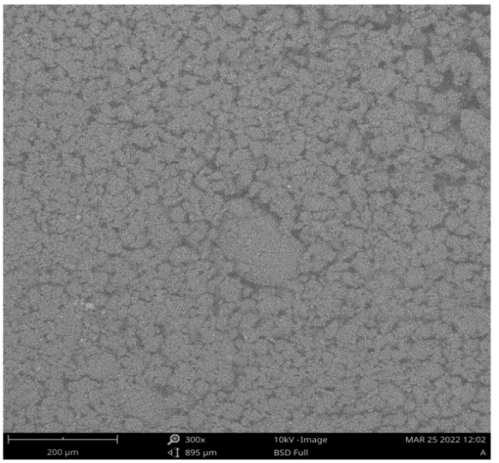

Plate 1: SEM Morphology of unmodified *Parkia biglobosa* at (X300) magnification

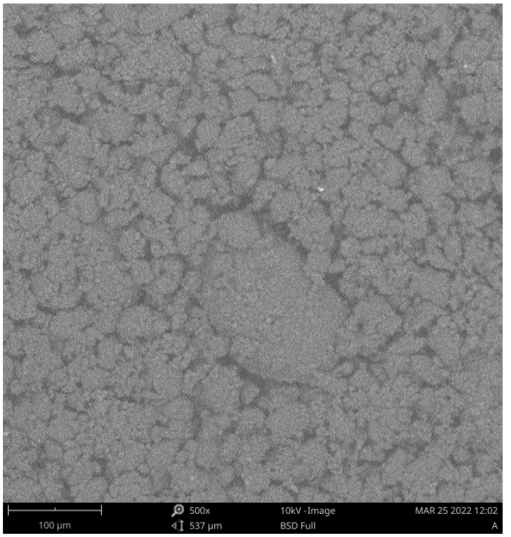

Plate 2: SEM Morphology of unmodified *Parkia biglobosa* at (X500) magnification

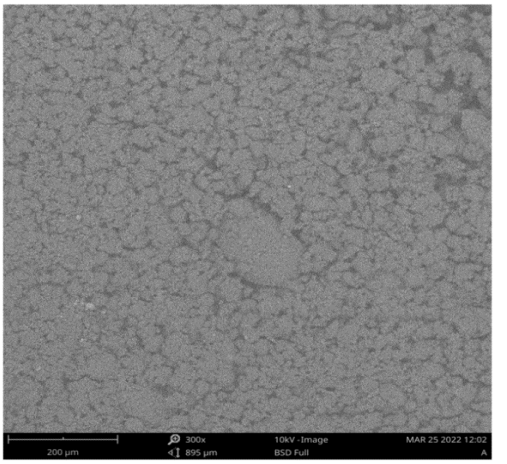

Plate 3: SEM Morphology of unmodified *Parkia biglobosa* at (X300) magnification

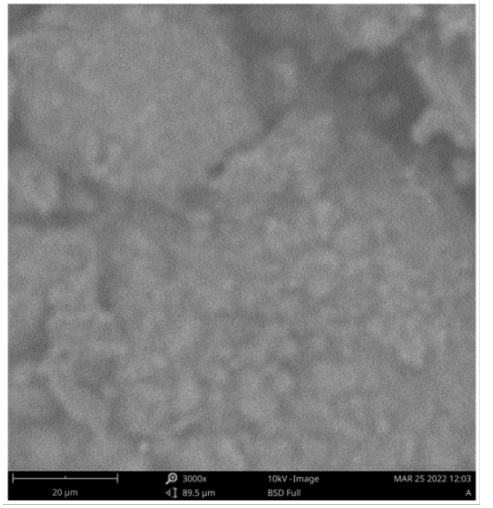

Plate 4: SEM Morphology of unmodified *Parkia biglobosa* at (X1500) magnification

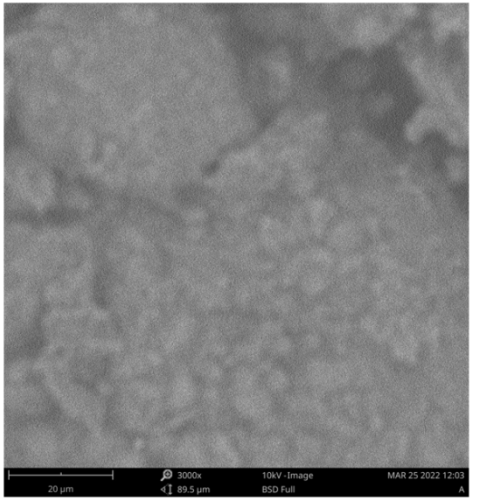

Plate 5: SEM Morphology of unmodified *Parkia biglobosa* at (X3000) magnification

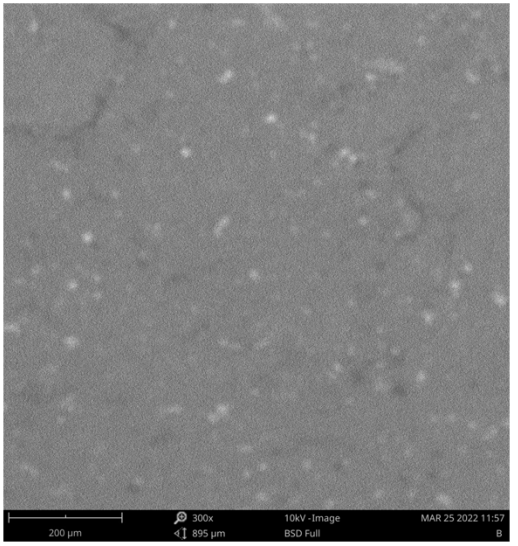

Plate 6: SEM Morphology of modified *Parkia biglobosa* at (X300) magnification

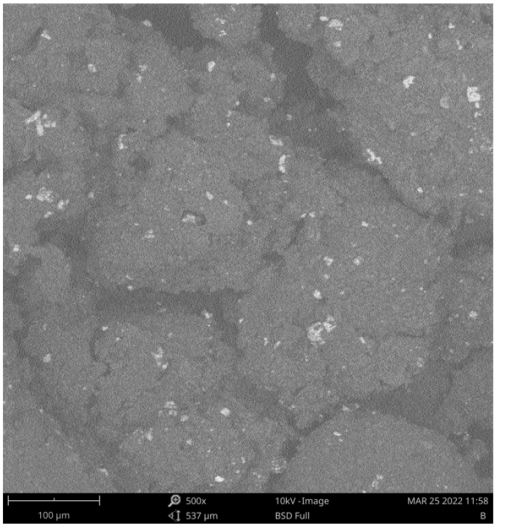

Plate 7: SEM Morphology of modified *Parkia biglobosa* at (X500) magnification

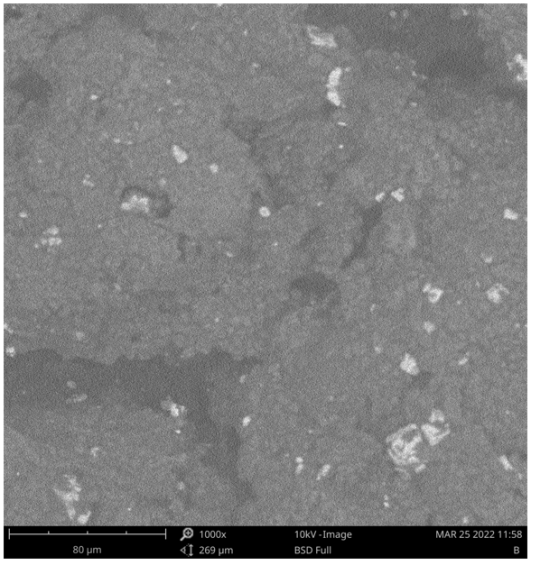

Plate 8: SEM Morphology of modified *Parkia biglobosa* at (X1000) magnification

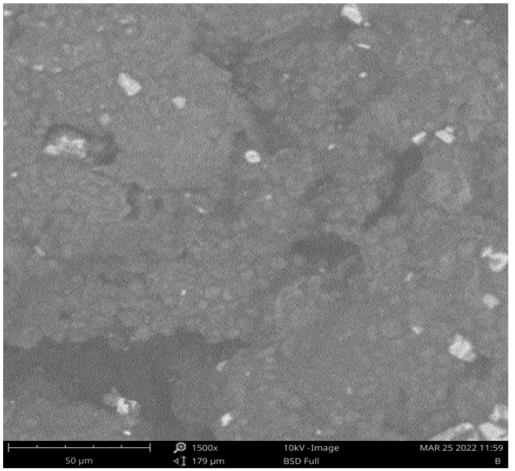

Plate 9: SEM Morphology of modified *Parkia biglobosa* at (X1500) magnification

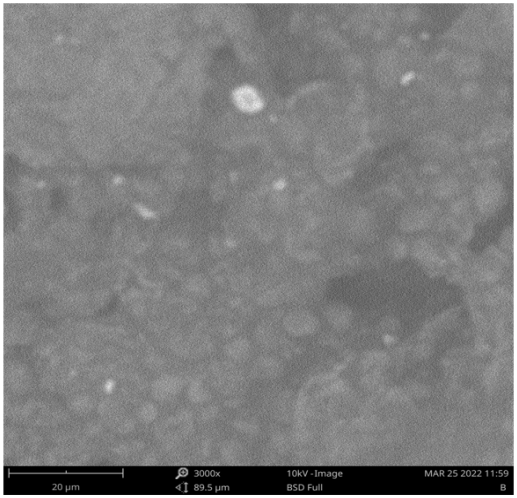

Plate 10: SEM Morphology of modified *Parkia biglobosa* at (X3000) magnification

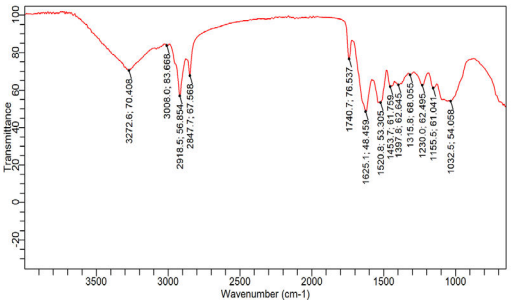

Fig. 1: FT-IR Spectrum of acetone based Extract of unmodified *Parkia biglobosa*

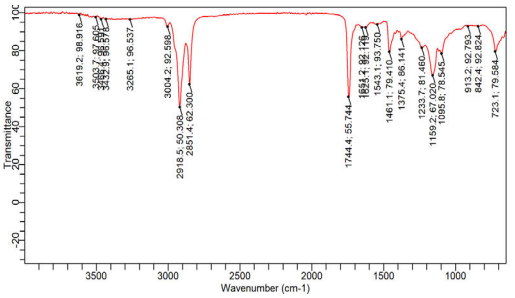

Fig. 2: FT-IR Spectrum of acetone based Extract of modified *Parkia biglobosa*

TABLE 1

Comparative table of functional groups intensities

FUNCTIONAL GROUPS	INTENSITIES (ACETONE MODIFIED)	INTENSITIES (UNMODIFIED)
O-H, N-H	96.59	70.47
C-H	92.11	67.56
N-H (in plane mode)	93.75	53.30
C-N (stretch)	79.42	68.05
SO_2 Sulphate (stretch)	NIL	61.04
C - CH_2 (scissors)	79.42	NIL
C=O	92.11	NIL
C=C	92.82	NIL
C - CH_3 (symmetrical bend)	86.14	NIL

Discussion

Plates (1-5) above show the morphological structure of unmodified *Parkia biglobosa* between (X300-3000/ magnifications; while plates (6-10) show the morphological structure of the modified *Parkia biglobosa* between (x300-3000) magnifications. As could be seen from the first five plates, there are evenly distributed patches with some pores in the unmodified SEM diagrams. However, the patches appear enlarged with scattered white patches in addition with pores. The enlargement of the patches is indicative of the fact that some molecules have been broken down from their initial forms e.g. proteins. A similar report has been reported by other researchers (Idika *et al*. 2023). Additionally, the white patches seen in plates (6-10) of the modified *Parkia biglobosa* could be attributed to diffusion of gases which could be one of the bye products of fermentation.

Figures (1 & 2) show the FT-IR scan of both the unmodified and modified acetone based extracts of *Parkia biglobosa*. Basically, the functional groups exhibited in the unmodified and modified acetone based extracts appear similar, but at varying proportions, with few appearing in the modified which were basically absent in the unmodified. However, in the intensities shown where the functional groups are similar, all appeared higher in the modified than the unmodified sample. These functional groups include O – H or N – H, C – H, C = O, strong N – H in a plane bend, C – N stretch and (SO_2 stretch) sulfate. But, the C – CH_2 scissors and C = C (benzene ring) were not present in the unmodified acetone based extract. Finally, (aromatic) C – N stretch in the unmodified extract was lacking in the modified extract. The importance of high intensity acetone based extract cannot be overemphasized due to its antimicrobial, anti-fungicidal and anti-bactericidal activities of its extracts (Fayinminnu *et al*. 2017) .

Despite the almost similar functional groups exhibited by both the unmodified and modified acetone extracts, the intensities of the functional groups found in the modified acetone extracts appear very large compared with its occurrence in the unmodified sample as shown in table 1.

This suggests that the antimicrobial, anti-fungicidal and the anti-bactericidal activities of the modified acetone-based extract could be more enhanced than in the unmodified acetone-based extracts.

Conclusion

This work identified similar and different functional groups in both the unmodified and modified *parkia biglobosa* acetone based extracts. However, the intensities found in the modified appeared bigger than the ones found in the unmodified extracts. This is suggestive of more microbial, fungicidal bactericidal activities in the modified acetone based extract over the unmodified extract.

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