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Phytochemical Composition and Functional Characterization of Parkia Biglobosa, *Moringa Oleifera*, and *Spondias Purpurea* Seeds from Adamawa North Senatorial Zone, Nigeria

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ABSTRACT

This study investigates the phytochemical composition and functional properties of *Parkia biglobosa*, *Moringa oleifera*, and *Spondias purpurea* seeds collected from Madagali, Michika and Mubi in Adamawa North Senatorial Zone of Adamawa State, Nigeria. High-Performance Liquid Chromatography (HPLC) analysis revealed significant antioxidant levels, particularly polyphenols and flavonoids, with *Moringa oleifera* exhibiting the highest antioxidant content. Fourier Transform Infrared Spectroscopy (FTIR) identified key functional groups, such as hydroxyl and carbonyl, associated with bioactivity. Scanning Electron Microscopy (SEM) revealed distinct morphological structures linked to functional attributes, suggesting potential for safe consumption. The seeds were further analyzed for bioactive compounds, dietary fiber, and antioxidant potential. Antioxidant activity, assessed via UV-Vis spectrophotometry and DPPH radical scavenging assays, demonstrated strong free radical scavenging abilities, particularly in *Spondias purpurea*. The study revealed that alkaloids were uniformly present (+) in all three seeds. Flavonoids were highest (+++) in *Parkia biglobosa* and *Moringa oleifera*, while *Spondias purpurea* had moderate (++) levels. Tannins were most abundant (+++) in *Spondias purpurea* compared to moderate (++) levels in the others. Saponins were abundant (+++) in *Parkia biglobosa* and *Spondias purpurea*, but lower (++) in *Moringa oleifera*. Terpenoids were moderate (++) in *Parkia biglobosa* and *Spondias purpurea* but high (+++) in *Moringa oleifera*. Glycosides were most abundant (+++) in *Moringa oleifera* and moderate (++) in others. *Moringa oleifera* had the highest IDF (93.76±3.44%), followed by *Spondias purpurea* (75.26±1.27%) and *Parkia biglobosa* (79.40±9.04%). *Parkia biglobosa* had the highest SDF (33.19±2.96%). The extracted IDF had greater WBC than crude seeds. IR spectra showed peaks at 3281.45, 2934, 1615, and 1030.90 cm^{-1}. Rutin ranged from 4.01–17.64 µg/g, while quercetin was 1.71–41.7 µg/g. These findings highlight the potential of these indigenous seeds as natural sources of antioxidants and dietary fiber, supporting their application in functional foods, nutraceuticals, and dietary supplements for improved health benefits.

Keywords: Phytochemical Screening, Antioxidant Activity, Functional Properties, Dietary Fiber, Indigenous Seeds.

INTRODUCTION

The development of medications, nutraceuticals, and functional foods all heavily rely on plant-based bioresources. As stores of bioactive substances such as flavonoids, polyphenols, and dietary fibers, seeds provide several health advantages, such as anti-inflammatory, antioxidant, and metal-binding properties [1], [2]. Because of their potential as a treatment and their contributions to sustainable agriculture and food security, it is imperative to investigate indigenous seeds.

In sub-Saharan Africa, underutilized seed resources with significant nutritional and therapeutic qualities include the African locust bean *(Parkia biglobosa*), the drumstick tree (*Moringa oleifera)*, and the red mombin (*Spondias purpurea*). Comprehensive phytochemical and functional characterizations are still scarce, despite their historical relevance [3], [4]. The identification of bioactive components and their industrial applications have been enhanced by developments in analytical techniques such as Scanning Electron Microscopy (SEM),

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Fourier Transform Infrared Spectroscopy (FTIR), and High-Performance Liquid Chromatography (HPLC) [5], [6]. In order to determine the safety and acceptability of plant materials for human consumption and therapeutic application, Atomic Absorption Spectroscopy (AAS) is also essential for determining the heavy metal level [2].

This study looks at the seeds of *Spondias purpurea*, *Moringa oleifera*, and *Parkia biglobosa* from Adamawa North Senatorial Zone, Adamawa State, Nigeria. With a focus on their antioxidant qualities and their uses in the food, nutraceutical, and pharmaceutical industries, it seeks to offer a thorough phytochemical and functional characterization. This study advances scientific knowledge of native plant resources through the use of cutting-edge analytical tools, promoting sustainable development and the advancement of world health.

In this study, the seeds of *Spondias purpurea*, *Moringa oleifera*, and *Parkia biglobosa* from Madagali, Michika and Mubi in Adamawa North Senatorial Zone in Adamawa State, Nigeria, were investigated. With a focus on their antioxidant qualities and their uses in the food, nutraceutical, and pharmaceutical industries, it seeks to offer a thorough phytochemical and functional characterization. This study advances scientific knowledge of native plant resources through the use of cutting-edge analytical tools, promoting sustainable development and the advancement of world health.

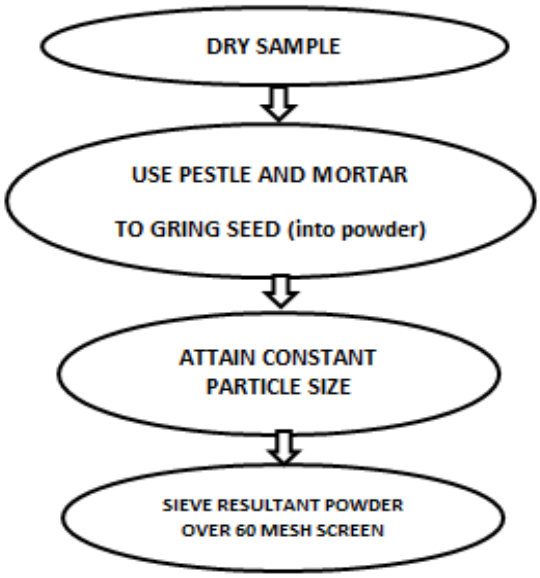
MATERIALS AND METHODS

Sample Collection and Preparation

Parkia biglobosa, *Moringa oleifera*, and *Spondias purpurea* seeds were collected from Madagali, Michika and Mubi of Adamawa North Senatorial Zone in Adamawa State, Nigeria. Here are some details about each of these places: Madagali: is a town within the Adamawa North Senatorial Zone that contribute to the rich cultural and historical tapestry of Adamawa State located along Latitude 10º53' 21"N, Longitude 13º37' 41"E with approximately population of 208,400 residents, Michika: is also a town situated along Latitude 10° 37' 0.0012" N, Longitude 13° 22' 59.9"E directly across the border from the famous tourist site of Mcedigyi in Vecemwe Rhumsiki with approximately 239,400 population. Mubi: one of the largest town after the state capital is a town with large economy that serves commercial area with Latitude 10.2676ºN, Longitude 13.2644ºE.

To guarantee precise identification, a botanist from the Department of Plant Science verified the species. To lower moisture levels and stop microbial growth, the seeds were carefully cleaned to remove dirt and contaminants before being allowed to air dry for seven to ten days at room temperature (25 to 30°C).

After drying, a pestle and mortar were used to grind the seeds into a fine powder. To attain a constant particle size, which is essential for reliable analytical results, the resultant powders were sieved over a 60-mesh screen (ISO 12099:2021).



Flow chart showing sample preparation process

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To maintain their quality and avoid oxidation or contamination, the produced samples were kept in airtight glass containers at room temperature. Following that, these samples were used for every phytochemical and physicochemical analysis.

Phytochemical Screening

Key bioactive substances, such as alkaloids, flavonoids, tannins, saponins, terpenoids, and glycosides, were identified using qualitative phytochemical analysis. The techniques used were predicated on the standardized procedures stated below by [7], [8] Mayer's and Dragendorff's reagents were used to detect the presence of alkaloids. Each reagent was applied separately to a tiny amount of the extract. The presence of alkaloids was verified by the production of an orange-red precipitate with Dragendorff's reagent or a cream-colored precipitate with Mayer's reagent.

The alkaline reagent test was used to test for flavonoids. A few drops of a 2% sodium hydroxide solution were added to the extract. Flavonoids were identified by a yellow tint that vanished when diluted hydrochloric acid was added. Key bioactive substances, such as alkaloids, flavonoids, tannins, saponins, terpenoids, and glycosides, were identified using qualitative phytochemical analysis. The techniques used were predicated on the standardized procedures stated below by [7], [8].

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Using the Keller-Kiliani test, glycosides were detected by treating the extract with glacial acetic acid that contained a few drops of ferric chloride solution, then carefully adding concentrated sulfuric acid. The presence of glycosides was confirmed when a blue-green color appeared in the acetic acid layer.

Extraction of Total Dietary Fiber

[9] Poised that, the enzymatic-gravimetric approach was used to extract the total dietary fiber, which comprises soluble dietary fibers (SDF) and insoluble dietary fibers (IDF). Alpha amylase (pH 1.5), pepsin (pH 6.8), and pancreatin (pH 4.5) were added to a 20 g portion of the sample suspended in sodium phosphate buffer (pH 6.0) and incubated for 1 hour each to eliminate protein and starch. Whatman filter paper without ash was then used to filter the enzyme digestate. The IDF was dried in an oven at roughly 37 degrees Celsius, chilled, and weighed after being thoroughly cleaned with ethanol and acetone.

By employing ethanol to precipitate the filtrate, the SDF was calculated. After being cleaned with ethanol and acetone, the precipitate was dried at 37°C, cooled, and weighed. The IDF and SDF seed samples' yield percentages were computed.

Water binding capacity (WBC)

A 0.5g portion of the sample was weighed into clean pre-weighed dried centrifuge tube and mixed with 7.5 mL distilled water. After 24h of equilibration at room temperature (approximately 25 °C), the suspension was centrifuged at 4,200 rpm for 15 min. Subsequently the supernatant was decanted and the tube with the sediment was weighed after removal of the adhering drops of water [10]. The water absorption capacity (WBC) was calculated using

$$\text{WBC}\left(\frac{\text{g}}{\text{g}}\right) = \frac{W_2 - W_1}{\text{Weight of Sample}}$$

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ANTIOXIDANT ANALYSIS

Chromatographic Conditions

A Diode Array Detector (DAD) tuned at 254 nm and an auto-sampler were included in the Agilent 1200 Series system used for the High-Performance Liquid Chromatography (HPLC) analysis. With a column temperature of 30°C and a run period of 25 minutes, the mobile phase flowed at 1.2 mL/min. Agilent (Eclipse) XDB-C18 (5 µm, 4.6 × 250 mm) was the column that was utilized, along with a C18 security guard column (4.0 × 3.0 mm).

Mobile Phase Preparation

20% acetonitrile and 80% 0.3% formic acid (V/V) made up the mobile phase, which was pH 3.0-adjusted with perchloric acid. Before use, the solution was sonicated for 30 minutes after passing over a 0.45 µm × 47 mm nylon membrane. [21]

Preparation of Diluent

The diluent was prepared by mixing equal volumes (50 mL) of HPLC-grade methanol and water, with pH adjusted using 1 mL glacial acetic acid. The solution was then sonicated for 30 minutes before use.

Rutin Standard Preparation

To create a 1 mg/mL stock solution, a 10 milligram quantity of the rutin reference standard was dissolved in methanol, sonicated for 10 minutes, and then diluted to 10 mL. A 0.2 µm × 13 mm syringe filter was used to filter the mixture. Using the diluent, serial dilutions (1.5625–100 µg/mL) were made.

Antioxidant Activity

[11] modified [12] approach and used the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay to assess the extracts' capacity to scavenge free radicals. 3 mL of extract solutions at several concentrations (50, 100, 200, 400, and 800 µg/mL) were combined with 1 mL of a pure 0.1 mM DPPH solution in methanol. After giving the concoctions a good shake, they were allowed to sit at room temperature for half an hour. A UV-VIS spectrophotometer was used to detect absorbance at 517 nm, using Trolox and gallic acid as reference standards.

The formula

$$\text{DPPH Scavenging Effecct (\%) or \% Inhibition} = \frac{A_{o-A_1}}{A_0} \times 100$$

Where A_0 is the absorbance of the control reaction and A_1 is the absorbance in the presence of the extract or reference standard, was used to determine the DPPH scavenging effect (%). Every experiment was carried out three times, and the outcomes were averaged. A log-dose inhibition curve was used to calculate the inhibition concentration (IC_{50}) value.

Attenuated total reflections (ATR) – Fourier transform infra – red (FTIR)

Analysis

The ATR-FTIR optical crystal, which includes KBR, was filled with roughly 20 mg of a dry sample that had been finely powdered. The PerkinElmer Spectrum model of ATR-FTIR was used to record the FTIR spectra at a resolution of 4 cm-1, covering the spectrum range of 4000 to 500 cm–1. In terms of hydroxyl, lipid, protein, and polysaccharide composition, the FTIR spectra mostly validate structural differences between the crude sample, insoluble dietary fiber, and soluble dietary fiber. The observed changes in intensity and shifts imply that fiber fractionation modifies the functional groups, especially when it comes to raising the amount of

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soluble fiber in SDF. These results are consistent with recent research that highlights how dietary fiber extraction affects structural and functional qualities [13].

Scanning electron microscope (SEM) analysis

The samples were analyzed using micrographs using the Phenom World model of scanning electron microscopy (SEM). On the silicon wafer, 30 mg of the sample particles were fixed, and they were then sputtered with gold until they were roughly 100 nm thick. The SEM was used to observe and document the sample's form and surface properties.

RESULT AND DISCUSSION

Phytochemical component of the seeds

Significant differences in the phytochemical profiles of *Parkia biglobosa*, *Moringa oleifera*, and *Spondias purpurea* seeds were found using phytochemical screening. These bioactive substances are crucial to their medicinal and functional qualities.The findings of the samples' phytochemical examination are displayed in Table 1 below:

Table 1: Using the methods described, the table below lists the bioactive chemicals that are present (+) or absent

(-) in *Parkia biglobosa*, *Moringa oleifera*, and *Spondias purpurea.*

Table 1: Parkia biglobosa, Moringa oleifera, and Spondias purpurea

Phytochemical Compound	***Parkia biglobosa***	***Moringa oleifera***	***Spondias purpurea***
Alkaloids	+	+	+
Flavonoids	+++	+++	++
Tannins	++	++	+++
Saponins	+++	++	+++
Terpenoids	++	+++	++
Glycosides	++	+++	++

Key: **+++**: Highly present

++: Moderately present

+: Slightly present

-: Absent

Alkaloids were uniformly present (+) in all three seeds. Alkaloids are known for their pharmacological properties, including analgesic, anti-inflammatory, and antimicrobial activities [14]. Their presence across all samples indicates potential for medicinal and nutraceutical applications.

Flavonoid content was highest (+++) in *Parkia biglobosa* and *Moringa oleifera* compared to moderate levels (++) in *Spondias purpurea*. Flavonoids are powerful antioxidants with roles in combating oxidative stress, reducing inflammation, and modulating cellular pathways [15]. The abundance in *Parkia biglobosa* and *Moringa oleifera* highlights their potential as natural antioxidant sources.

Tannins were most abundant (+++) in *Spondias purpurea* compared to moderate levels (++) in the other two species. Tannins possess astringent, antimicrobial, and anti-inflammatory properties, making *Spondias purpurea* particularly useful in applications like wound healing and gastrointestinal protection [16].

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Saponins were abundant (+++) in *Parkia biglobosa and Spondias purpurea*, but relatively lower (++) in *Moringa oleifera*. These compounds are known for their cholesterol-lowering, immune-boosting, and anticancer properties [17]. The higher levels in *Parkia biglobosa* and *Spondias purpurea* enhance their therapeutic potential.

Parkia biglobosa and Spondias purpurea showed moderate (++), while *Moringa oleifera* exhibited high (+++) levels of terpenoids. Terpenoids are known for their antimicrobial, anti-inflammatory, and anticancer effects [18]. The high content in *Moringa oleifera* supports its traditional use in medicine.

Glycosides were most abundant (+++) in *Moringa oleifera*, followed by moderate (++) levels in the other seeds. Glycosides have cardioprotective, laxative, and anticancer activities. The high content in *Moringa oleifera* aligns with its diverse health applications

Dietary Fiber content

Parkia biglobosa, Moringa oleifera, and *Spondias purpurea* were the three seed samples used for the investigation, and the % yield of insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) was assessed. The fiber composition of these samples varied significantly, according to the results, with *Moringa oleifer*a having the greatest IDF concentration, followed by Parkia biglobosa and *Spondias purpurea.* On the other hand, Spondias purpurea and *Moringa oleifera* displayed lesser SDF content, whilst Parkia biglobosa had the greatest.

Table 2: Dietary fiber content of the samples of studies

Sample	% Yield IDF	% Yield SDF
Pakia biglobasa	79.40±9.04	33.19±2.96
Moringa oleifera	93.76±3.44	25.59±1.79
Spondius purpura	75.26±1.27	21.07±0.91

Moringa oleifera has the greatest IDF content (93.76±3.44%), followed by *Spondias purpurea* (75.26±1.27%) and *Parkia biglobosa* (79.40±9.04%). By encouraging bowel movements, lowering constipation, and binding toxins for elimination, insoluble fiber is essential for improving intestinal health [19]. Moringa oleifera's high IDF content raises the possibility that it could be a beneficial dietary fiber source for gut health. Furthermore, *Parkia biglobosa* and *Spondias purpurea's* comparatively high IDF output suggests that they could be used in dietary formulations that enhance gut motility and digestion.

Parkia biglobosa had the greatest SDF content (33.19±2.96%), followed by *Spondias purpurea* (21.07±0.91%) and *Moringa oleifera* (25.59±1.79%). Lowering cholesterol, controlling blood sugar, and fostering a healthy gut microbiome are all documented benefits of soluble fiber [9]. *Parkia biglobosa's* comparatively large SDF yield points to the possibility of using it in functional foods that promote metabolic and cardiovascular health.

Water binding capacity (WBC)

The crude seed samples' water binding capacity (WBC) and the insoluble dietary fibers (IDF) that were isolated from them were examined. The WBC is a crucial functional characteristic that affects texture, shelf stability, and satiety effects in food applications by determining the dietary fiber's capacity to absorb and hold onto water [10].

The findings show that in every case, the extracted IDF had greater WBC than the crude seeds. In particular, *Parkia biglobosa, Spondias purpurea,* and *Moringa oleifera* IDF showed the greatest WBC as seen in the following figure 1.

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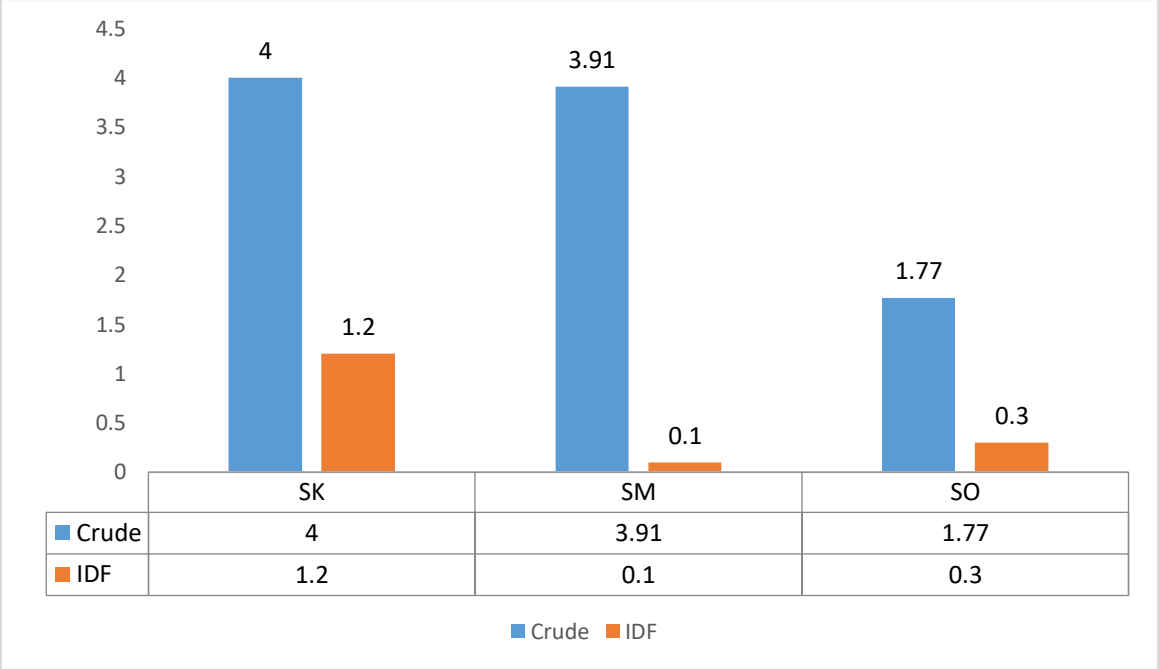
Figure 1: Water Binding Capacity of the seed samples



Key: IDF = Insoluble dietary fiber; *Pakia biglobasa (***SK***),*

Moringa oleifera **(SM)** *and Spondius purpure* **(SO)**

Because more hydrophilic areas are exposed as a result of the ablation of non-fibrous components, IDF has improved water retention. According to these results, the extracted IDF may be useful in food compositions that need to retain a lot of water, like meat extenders, bread goods, and functional health foods. These fibers are advantageous for weight control and gut health applications since the elevated WBC may also lead to better satiety and digestive health.

Table 3: Rutin Content, Quercetin Content and the IC_{50} values in the Seed Samples

Sample Code	**Rutin Content (µg/g)**	**Quercetin Content (µg/g)**	**IC_{50} (µg/mL)**
Pakia biglobasa	7.94±0.78	1.79±0.19	653.87
Moringa oleifera	4.01±0.01	1.71±0.54	228.94
Spondius purpure	17.64±0.13	41.70±12.84	654.76
Gallic Acid (Positive control)	NA	NA	36.49
Trolox (Positive control)	NA	NA	11.84

Results are presented in Mean ± SD

NA = Not Applicable

The study evaluated the antioxidant capacity of seeds from *Spondias purpurea*, *Moringa oleifera*, and *Parkia biglobosa* by contrasting them with Trolox and gallic acid. The antioxidant activity of *Spondias purpurea* was the highest (17.64±0.13, 41.70±12.84, 654.76), outperforming *Moringa oleifera* (4.01±0.01, 1.71±0.54, 228.94) and *Parkia biglobosa* (7.94±0.78, 1.79±0.19, 653.87). Its high level of flavonoids and polyphenols, which are known to scavenge free radicals, may be the cause of this [20].

Because of its tannins, flavonoids, and alkaloids, *Parkia biglobosa* exhibited modest antioxidant activity. According to [19], *Moringa oleifera* showed lower antioxidant levels, which is consistent with reports that its leaves contain more antioxidant chemicals than its seeds. *Spondias purpurea* outperformed Trolox, indicating its promise as a natural antioxidant source for food and medications, even though none of the seeds could equal gallic acid's antioxidant capability.

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ATR – FTIR analysis

The IR spectra for the crude of the studied samples and their insoluble dietary fiber (IDF) and soluble dietary (SDF), are presented in Fig. 2-4 below:

Fig 2: FTIR Spectra of Crude Pakia biglobasa (SK) and its Insoluble Dietary Fiber (IDF) and Soluble Dietary Fiber (SDF)

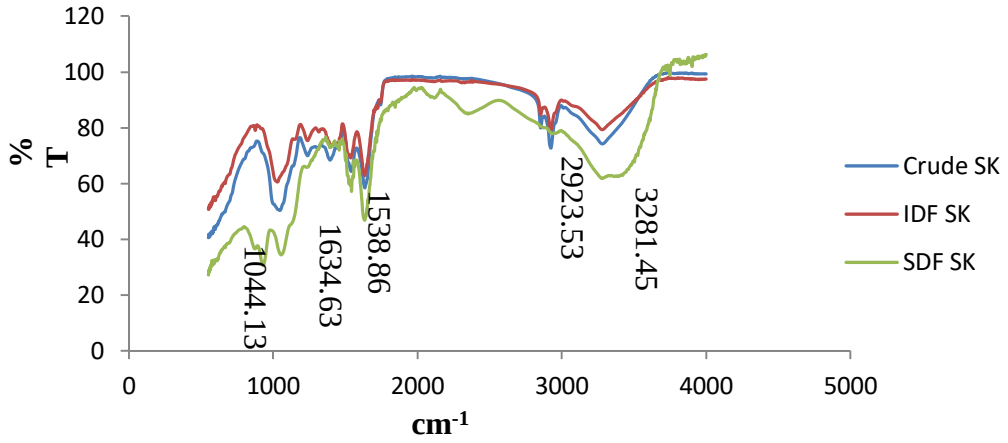


Figure 3: FTIR Spectra of Crude *moringa oleifera* (SM)and its Insoluble Dietary Fiber (IDF) and Soluble Dietary Fiber (SDF)

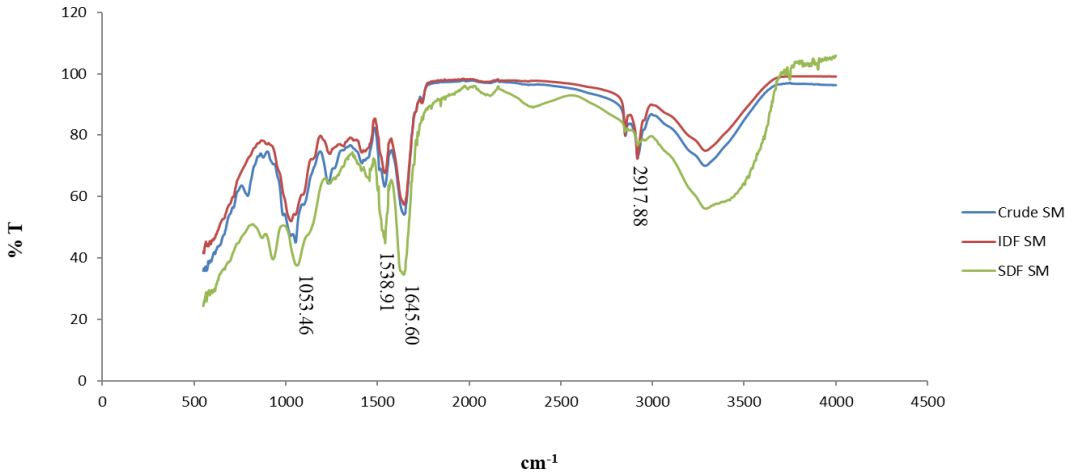
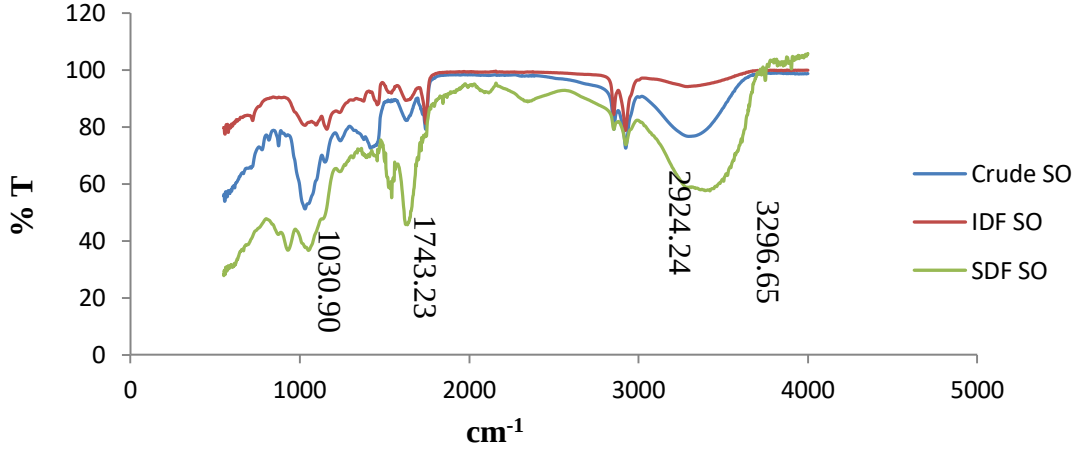


Figure 4: FTIR Spectra of Crude *Spondius purpure* (SO) and its Insoluble Dietary Fiber (IDF) and Soluble Dietary Fiber (SDF)



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Scanning electron microscope (SEM) analysis

Analysis using a scanning electron microscope (SEM) Figures 5 to 7 (a) (b) and (c) accordingly display the findings of the scanning electron microscopy (SEM) micrograph of the crude samples, their IDF, and their SDF.

SEM of crude *Pakia biglobasa* (a), along with its insoluble dietary fiber (IDF) (b), and soluble dietary fiber (SDF) (c) and is shown in Figure 5.

Figure 5: SEM of Crude *Pakia biglobasa* (a) and its Insoluble Dietary Fiber (IDF) (b) and Soluble Dietary Fiber (SDF)

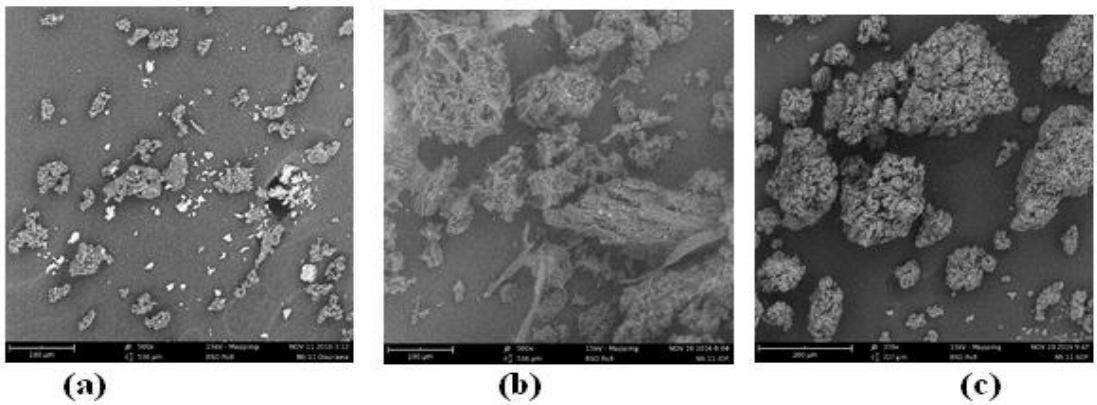


Figure 6: SEM of Crude *Moringa oleifera* (a) and its Insoluble Dietary Fiber (IDF) (b) and Soluble Dietary Fiber (SDF) (c)

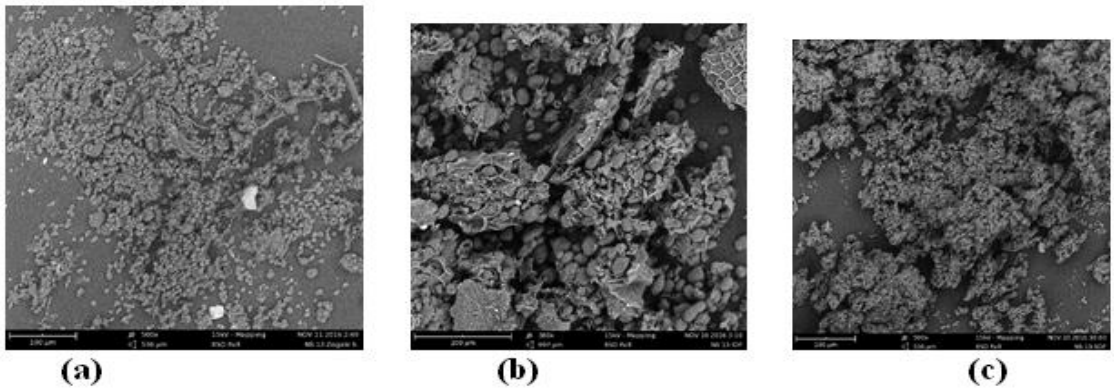
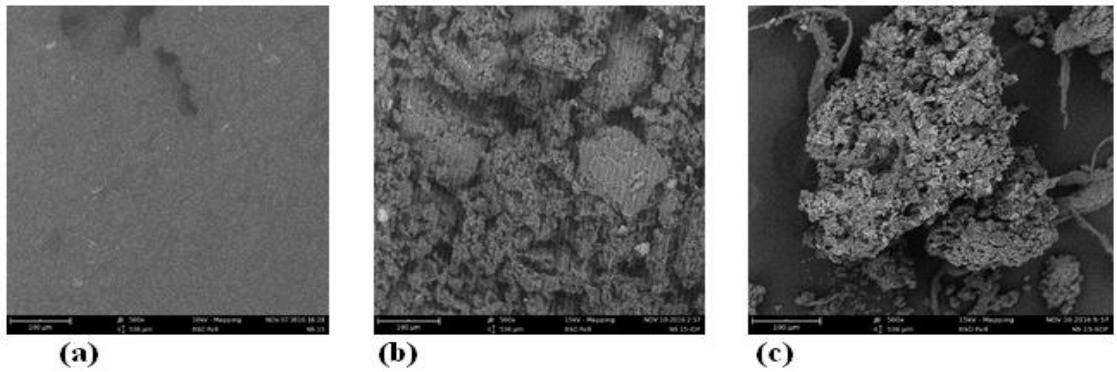


Figure 7: SEM of Crude *Spondius purpura (SO)* **(**a) and its Insoluble Dietary Fiber (IDF) (b) and Soluble Dietary Fiber (SDF) (c)



The fibrous and rough surface of crude *Moringa oleifera* powder indicates the presence of cellulose, hemicellulose, and lignin [11]. The high lignocellulosic content of *Parkia biglobosa* seeds results in a dense,

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stiff structure that affects extraction efficiency and digestibility. Given its high polysaccharide content, *Spondias purpurea* seeds have a tight matrix with fibrous threads and embedded starch granules [4]. While *Parkia biglobosa* IDF possesses elongated, thick fibers that facilitate gastrointestinal motility and cholesterol binding [17], *Moringa oleifera* IDF produces a porous, fibrous network that improves edema and water retention [13]. The rough surfaces and fragmented structure of *Spondias purpurea* IDF contribute to its capacity to absorb water and oil.

Fiber deterioration is noticeable in SDF fractions. The disordered, asymmetrical structure of *Moringa oleifera* SDF promotes gel-forming properties [15]. The porous and fractured nature of *Parkia biglobosa* SDF enhances emulsification and digestibility [11]. The sponge-like, extremely porous network of *Spondias purpurea* SDF is advantageous for the encapsulation of bioactive compounds in nutraceuticals [18].

CONCLUSION AND RECOMMENDATIONS

Based on the findings of this study, it is recommended that *Parkia biglobosa*, *Moringa oleifera*, and *Spondias purpurea* seeds be further explored for their potential applications in food, pharmaceuticals, and nutraceuticals due to their high antioxidant content, bioactive compounds, and functional properties. *Given Moringa oleifera's* superior antioxidant profile and *Spondias purpurea's s*trong free radical scavenging capacity, these seeds could be valuable natural sources for enhancing dietary health and preventing oxidative stress-related diseases. Further studies on extraction techniques, bioavailability, and industrial applications are encouraged to maximize their potential benefits.

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