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Extraction and Characterization of Natural Dye Obtained from African Locust Bean (*Parkia biglobosa*) Pod Bark

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Abstract

Although natural dyes have attracted growing interest as sustainable alternatives to synthetic colorants, studies on the physicochemical, phytochemical, antimicrobial, and structural properties of dye extracts from African locust bean pod remain limited. This study investigated the extraction and characterization of a natural dye from African locust bean pod using the maceration method. The extraction yielded 23.76%, and the dye extract was dark brown, with a pH of 5.77, a melting point of 440 °C, a density of 0.54 g/mL, partial solubility in water, and complete solubility in methanol. Phytochemical screening revealed the presence of tannins, flavonoids, alkaloids, glycosides, phlabotannins, anthraquinones, quinones, terpenoids, steroids, carbohydrate, starch, proteins, and anthocyanins, while saponin was absent. The extract demonstrated antibacterial activity against *B. subtilis*, *E. coli*, *S. aureus*, *P. aeruginosa*, and *S. typhi*, and antifungal activity against *P. notatum*, but no antifungal activity was observed against *C. albicans* and *A. niger* at all concentrations. Characterization was conducted using UV-Vis, FTIR, GC-MS, HPLC, and SEM analyses. The UV-Vis spectrum showed distinct absorption

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peaks, with the highest absorbance recorded at 287 nm (4.1000), likely associated with carbonyl (C=O) groups characteristic of hydrolysable tannins. FTIR analysis indicated the presence of C–H stretching, C=C stretching, C=O stretching, and C–O stretching functional groups. GC-MS identified 19 compounds with varying retention times and peak areas, among which cis-9-hexadecenal (14.81%) was the major compound, followed by heneicosanoic acid (8.59%), hexadecanoic acid (7.93%), and octadecanoic acid (7.31%). HPLC analysis revealed gallic acid as the most abundant compound, indicating the presence of hydrolysable tannin, while SEM showed a rough and porous surface morphology with a compacted, fused-like solid structure. These findings demonstrate that African locust bean pod is a promising source of natural dye with notable bioactive and structural characteristics, thereby contributing to the growing body of knowledge on plant-based dye materials.

Keywords: African Locust Bean Pod; Natural Dye; Phytochemical Screening; Antimicrobial Activity; Characterization

INTRODUCTION

Colors offer an important window into our reality. They enhance the appeal of common materials like paints, fabrics, plastics, paper, and food. A colored material with an affinity for the surface it is applied on, is called a dye (Kumar *et al*., 2021). Natural dyes are substances from plants and animals that impart color to foods, cosmetics, drugs, hair, fiber, fur and polymers. Natural dyes may be obtained from several plant parts like flowers, roots, leaves, bark, insects' secretion and minerals. Although dyes are abundant in the natural environment, they can also be formed in different ways and used in different applications depending on their manufacturing processes (Nwoye and Ezema, 2017).

Environmental contamination is now a global problem. Since effluents contaminate water, the textile sector is reported to be among the most polluting. Toxic substances are included in these effluents. The unrestrained use of synthetic dyes in the textile sector usually worries environmentalists since it pollutes water and causes other waste disposal issues. Additionally, studies have demonstrated that the majority of synthetic dyes harm the body by reducing growth and fertility, reducing the ability to consume food, damaging the liver, spleen, kidney, and heart, and causing lesions on the skin, eyes, lungs, and bones (Patil et al., 2019).

Numerous plant species found on the African continent have the potential to yield new natural products with dye-yielding properties (Wanyama *et al.*, 2014). Nigeria has abundant resources in terms of plants which contain dyes, they are found in leaves, bark, roots, seeds, fruits and flowers (Nwoye and Ezema, 2017). Most of west Africa, particularly northern Nigeria, discards the locust bean fruit (pods) after de-hauling the empty pods, which has a detrimental impact on the ecosystem. This research is focused the extraction, physical properties, antimicrobial, phytochemical screening and characterization of the dye extract from African locust bean pod bark.

Plate 1: Dried pod of African Locust Bean

MATERIALS AND METHODS

Sample Preparation

Fresh pod of locust bean *Parkia biglobosa* was collected from Gamboru in Maiduguri metropolitan, Borno State. Then the pod bark was chopped into tiny pieces, dried at room temperature, crushed into powder.

Extraction and Optimization of the Dye

A container containing 200 g of the powdered stuff was filled with 500 ml of methanol. For efficient extraction, the mixture was left to stand for a week. Following that, it was filtered, the filtrate was dried off, and the solute was gathered and weighed. Optimization was carried out at different time (Leonard et al., 2022).

Physical Properties of the Dye extract

Determination of Melting Point of the Dye extract

To determine the melting point, 0.1g of the locust bean pod's solid dye extract was added to the capillary tube, which was then placed within the melting point apparatus. The temperature was steadily increased and closely monitored until melting began. It was noted what temperature the melting started at and the readings were recorded (Musa et al., 2018).

Solubility Test of the Dye extract

Zero-point one gram of the dye extract was used in the solubility tests of the dye material in 1 ml of cold water (Egbujor *et al.,* 2023).

Determination of pH of the Dye extract

Twenty milliliters of deionized water were added to a 100 ml beaker containing 1 g of the dye extract. After then, the mixture was agitated for several minutes. The mixture was allowed to stand for ten minutes. The dye sample and the buffer were both let to reach room temperature. A pH 4 buffer solution was used to calibrate the pH tester. After rinsing with deionized water, the electrode and automated temperature compensation (ATC) were blotted dry. After submerging the electrode in the dye sample solution, the pH was determined and noted (Egbujor *et al.,* 2023).

Determination of color of the Dye extract

The color of the dye extract was determined by ordinary physical observation (Egbujor et al., 2023).

Determination of Density of the Dye extract

Density was carried out using standard test methods (Egbujor *et al.,* 2023).

Phytochemical Screening of the Dye

The dye extract was subjected to a phytochemical test as described by Banu and Cathrine (2015), Balamurugan *et al.* (2019).

Test for Tannins

A few drops of FeCl3 solution were added after 2 ml of the dye extract and 2 ml of distilled water had been mixed. The presence of tannins is shown by the production of a green precipitate.

Test for Saponins

In a test tube, about 5 ml of the dye extract was heated after being vigorously mixed with 5 ml of distilled water. Stable formation of foam was interpreted as a sign that saponins were present.

Test for Phlobatannins

After adding 2 ml of the dye extract to 2 ml of 1% HCl, the liquid was brought to a boil. A red precipitate's deposition was interpreted as confirmation that phlobatannins were present.

Test for Flavonoids

One milliliter of a 10% lead acetate solution was added to 1 ml of the dye extract. A yellow precipitate's development was interpreted as a flavonoid test result. When concentrated H_2SO_4 was added to the dye, an orange color formed, indicating the presence of flavonoids.

Tests for Anthraquinones

Five milliliters of a 10% ammonia solution was added to the filtrate, after 3 ml of the dye extract and 3 ml of benzene were agitated and filtered. After shaking the mixture, the existence of free anthraquinones is indicated by the ammonical (lower) phase turning pink, red, or violet.

Test for Quinones

When 1ml of alcoholic KOH was added to 1 ml of dye extract, the color changed from red to blue, indicating the presence of quinones.

Test for Terpenoids

Two milliliters of the dye extract was dissolved in 2 ml of chloroform and then dried by evaporation. After that, 2 ml of concentrated sulfuric acid was added, and the mixture was heated for two minutes. The presence of terpenoids is indicated by a greyish color.

Tests for Steroids

When 2 ml of dye extract was dissolved in 2 ml of chloroform and 2 ml of concentrated sulfuric acid was added, a red color appears in the lower chloroform layer, indicating the presence of steroid

Test for Alkaloids

The presence of alkaloids is shown by a reddish-brown precipitate that forms, when a 3 drops of Wagner's reagent was added to 1 ml of dye extract.

Test for Carbohydrates

About 0.5 milliliters of Benedict's reagent was added to 0.5 milliliters of the dye extract. In a boiling water bath, this combination was heated for two minutes. The presence of sugars is indicated by the formation of deep red precipitate.

Test for Starch

About 2 ml of the dye extract was mixed with 0.01 g of iodine and 0.075 g of potassium iodide in approximately 5 ml of distilled water. The development of a blue color indicates the presence of starch.

Test for Glycosides

Two milliliters of dye extract was dissolved in 2 ml of chloroform. After carefully adding 2 ml of sulfuric acid, the mixture was gently shaken. The presence of a steriodal ring, or the glycone part of a glycoside, is indicated by a reddish-brown color.

Test for proteins

Two milliliters of dye extract was mixed with one drop of a 2% copper sulphate solution, followed by 1 ml of 95% ethanol. After then, excess KOH was added. The presence of protein is indicated by the appearance of pink color.

Test for anthocyanins

The presence of anthocyanins is indicated by the color changing from pink-red to blue-violet when 2 ml of HCl and 2 ml of ammonia were added to 2 ml of the dye extract.

Test for Coumarins

Two milliliters of the dye extract was mixed with 3 ml of a 10% aqueous solution of NaOH. The presence of coumarins is indicated by the appearance of yellow color.

Screening for Antimicrobial Activity of the Dye extract

Determination of Zone of Inhibition

The dye extract's antimicrobial properties were tested using the hole-in-plate disc diffusion technique. After boring wells in the media with a 6 mm cork borer, 0.2 mL

aliquots of the extracts at various concentrations were put within. The agar plate was then incubated at 37 ºC for 24 hours. Following incubation, a transparent meter rule was used to measure the diameters of each extract's zone of inhibition in millimeters. Every extract was examined three times (Usman *et al.*, 2018).

Determination of Minimum Inhibitory Concentration (MIC)

The extracts were serially diluted to obtain concentrations of 6.25, 12.5, 25, 50, and 100 mg/ml in order to determine the MIC. In nutrient broth, the bacterial strains were cultivated, then 1.0 ml of test concentrations of each were added to a 1.0 ml microbe suspension in the microplate, which was then incubated for 24 hours at 37 oC (Afolabi et al., 2020).

Determination of Minimum Bactericidal Concentration (MBC)

The suspensions from the MIC experiments were used to calculate the MBC. To produce a bacterial streak, equal streaks of the MIC suspension were applied to a solid medium. This procedure was repeated until the required quantity of matched isolates was obtained. The concentrations were incubated at 37 oC for 24 hours. The plates were inspected after the incubation time (Afolabi et al., 2020).

Ultraviolet/Visible Analysis

A UV/Visible spectrophotometer was used to analyzed a diluted dye solution that had been added to a quartz cell with a path length of 1cm. Ethanol served as the blank dilution solvent. To create the sample's distinctive absorption spectra, a scan from 200 to 700 nm was conducted, and the maximum absorption (λmax) was calculated (Egbujor *et al.,* 2023).

Fourier Transform Infrared (FTIR) Analysis

Wavenumbers ranging from 4000 to 650 cm^{-1} were employed in the transmittance approach. The dye extract was subjected to infrared (IR) spectroscopy, and its absorption spectra was obtained. The peaks in the spectra corresponded to specific frequencies ($cm^{-1)}$. Each peak in the spectra was interpreted and the possible functional groups were identified (FTIR-8400S) (Nnorom and Onuegbu, 2019).

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The Agilent Tech GC 7890B, MSD 5977A, and mass detector were used to perform GC-MS analysis on the dye extract. Helium was utilized as the carrier gas at a flow

rate of 1 ml in 1 min, and 1μL of the sample's supernatant was fed into the GC. The GC oven was set to begin at 80 °C and increase in temperature by 15 °C per min to 200 °C, then by 5 °C per min to 280 °C, reaching a 5-min isothermal at 280 °C. The ion source was adjusted at 230 °C, and the ionization voltage was adjusted at 70 eV (Ibrahim *et al.,* 2021).

Dye Separation and Identification Using High Pressure Liquid Chromatography (HPLC)

HPLC separation was achieved with an Agilent 1260 series equipped with a 1260 Quat pump VL quaternary pump, a 1260 ALS autosampler, a 1260 TCC column thermostat, and a 1260DAD VL diode array detector. The separation was carried out using a Hypersil BDS C18 column (4.6 x 100 mm i.d., 3.5 μm) and a C18 guard column (4 x 10 mm i.d., 3 μm). The mobile phases used in gradient elution were (A) methanol and (B) 0.5% acetic acid in water. Before the start of each investigation, this column was re-equilibrated with 10% B in A for 10 minutes, and the flow rate was fixed at 1.0 ml/min while the temperature was controlled at 25 °C. The DAD detector was set at a wavelength of 326 nm, and each sample and reference had an injection volume of 5 μl (Nkwocha et al., 2022).

Scanning Electron Microscope (SEM) Analysis of the Dye extract

A Quanta 250, FEI, Netherlands Scanning Electron Microscope was used to examine the dimensions and form of sample at various stages. The sample was placed on aluminum stubs using conductive carbon tape, and then it was sputtered with gold using a plasma sputtering equipment under vacuum at 20mA for two minutes. The sample was then be inspected and captured on cameras (Cheran et al., 2022).

RESULTS AND DISCUSSION

Extraction and optimization of the Dye extracts from the Locust bean pod

Table 1 shows the percentage yields of the dye. The percentage yields extracted for 24 hours was (7.8%), 72 hours was (8%), for a week was (23.76%), From the results, it was observed that the yield increased with longer extraction times and decreased with shorter extraction. Leonard et al., (2022) reported that the percentage yield of 6.3% (12.93 yield) from the ethanol dye extract of parkia biglobosa tree bark after a week extraction.

Table 1: Percentage yield of Dye extracts

S/N	Solvents	Extraction Time	Yield (g)	% Yield
1.	Methanol	24 hours	15.60	7.80
		72 hours	16.00	8.00
		1 week	47.52	23.76

Physical Properties of the Locust Bean Pod Dye extracts

Table 2 shows the physical properties of the dye extracts. The melting point of a substance is the temperature at which it changes from a solid to a liquid. MLBP had melting point of 44 oC. These low melting points may likely be due to weak molecular forces like Vander Waals interaction between molecules in their chemical structure, which requires less energy to overcome and transition from solid to liquid state (Shruti et al., 2018). Carrot root dye extract melted at 34 oC, turmeric root dye extract melted at 46 oC, and onion bulb dye extract melted at 26 oC (Egbujor et al., 2023).

Solubility is the ability of a chemical compound (referred to as the solute) to dissolve in a solvent (usually a liquid) and form a solution. The MLBP dye extract is partially soluble in water. This is because the polarity of methanol is similar to water. The OH group in methanol allows hydrogen bond with other polar compounds enhancing solubility. Therefore, compounds extracted by methanol are hydrophilic (strong affinity for water) are more likely to be soluble (Rajhard et al., 2021). The dye material from onion bulbs was found to be soluble in water. Carrot dye and tumeric dye extract were also soluble in water (Egbujor et al., 2023). Leonard et al. 2022 reported that the ethanol dye of parkia biglobosa tree bark is soluble in water.

The MLBP dye extract has a pH of 5.77 which suggest that the dye extract is slightly acidic. The dye extract is acidic. This acidity could be due to the presence of functional groups like sulfonic or carboxylic acid present in the structure of the dye extracts, which readily releases hydrogen ions (Pal, 2017). The pH values obtained from the dyes extracts made from onion bulbs, carrots, and turmeric roots were 4.04, 4.57, and 5.25, respectively. Since all of the collected pH values fall on the left side of the pH scale, it was evident that all the dyes were acidic (Egbujor et al., 2023).

The dark brown color of the MLBP dye extract suggests the presence of chromophores within the locust bean Parkia biglobosa pod bark. These compounds, likely derived from tannins are responsible for the color intensity. The colors of dyes obtained from plants are as a result of the presence of flavonoids, tannins and other phytochemicals.

Hence, a single compound is not responsible for the color (Yadav et al., 2023). Egbujor et al. (2023) reported that, onion bulb dye was dark tan, carrot root dye was dark red and turmeric root dye was dark orange. Leonard et al. 2022 reported that the ethanol dye extract of parkia biglobosa tree bark is dark brown in color.

The density of the MLBP dye extract is 0.54 g/mL, which indicates that the dye is relatively lightweight compared to water (density of 1 g/mL). This could be due to the presence of organic compounds or air pockets within the dye.

Table 2: Physical Properties of the Dye Extracts

S/N	Properties	MLBP
1	Melting Point (^oC)	44
2	Solubility in water	Soluble
3	pH Value	5.77
4	Color	Dark brown
5	Density(g/cm^3)	0.54

Keys: MLBP =Methanol dye extract,

Phytochemical Screening of the Dye extracts from Locust Bean Pod

The qualitative phytochemical investigation of the methanol dye extracts, as shown in Table 3, revealed that tannins, quinones, terpenoids, alkaloids, glyscosides, flavonoids, anthraquinones, steroids, phlobatannins, anthocyanins, carbohydrate, starch and protein were found. Saponins and cumarines were absent. Verma et al., (2018), reported that tannins, flavonoids, anthraquinones, glycosides, and terpenoids were found in Onion peel methanol and ethanol dye extracts. Gracelin and Kumar, 2023 also reported that alkaloids, tannins, anthrquinones, glycosides, and carbohydrates were found in the methanol dye extract from Lawsonia inermis L. Anthrquinones, phlobatannins, alkaloids, and carbohydrates were also found in the dye extract from Beta vulgaris L. Terpenoids, alkaloids, tannins, flavonoids and steroids were reported to be found in the methanol dye extract of Hibiscus sabdariffa L. (Baryyah et al., 2019). The presence of tannins, steriods, flavonoids, and carbohydrates in the methanol dye extract from Curcuma longa L. (Irshad et al., 2018). The phytochemical screening of celosia cristata stem dye showed that terpenoids, flavonoids, tannins, and steroids were found (Sishakala et al., 2024).

Table 3: Qualitative Phytochemical Screening of the dye extracts

S/N	Phytochemicals	M.LBP Extract
1	Alkaloids	+
2	Flavonoids	+
3	Tannins	+
4	Saponins	_
5	Glycosides	+
6	Phlobatannins	+
7	Anthraquinones	+
8	Quinones	+
9	Terpenoids	+
10	Steroids	+
11	Carbohydrates	+
12	Starch	+
13	Proteins	+
14	Anthocyanins	+
15	Cumarines	_

Keys: + = Present - = Absent

Antimicrobial Activity of the Methanol Dye extract

Zone of Inhibition

The dye extract showed antibacterial activity against B.subtilis, with the highest concentration (100 mg/mL) showing the largest zone of inhibition of 16.00±0.00a as presented in Table 4. This indicates that the dye may be effective against this bacterium. The dyed was also effective against E. coli (12.00±0.00a), S. aureus (10.00±0.00a), P. aeruginosa (12.00±0.00a) and S. typhi (14.00±0.00a) at 100 mg/mL but less compared to B.subtilis. Dye extract from Swietenia macrophylla was also reported to have a similar zone of inhibition of 10.00±1.00 against S. aureus (Akhtar et al., 2022). The activity of methanol dye extract of Lawsonia inermis against P. aeruginosa, exhibited maximum activity at 75% (4.6±3.16mm) followed by 100% (3.3±2.16mm), then 50% (2.8±2.4mm) and 25% (2.6±2.1mm) (Kannahi and Vinotha, 2013). The antibacterial activity of the microbes increases with increase in concentrations of the dye, indicating a concentration-dependent effect. Ciprofloxacin (5mg/mL) was used as bacterium positive control.

Table 4: Antibacterial Activity of the Methanol Dye extract

	Extract concentration (mg/mL)/Zone of inhibition (mm)mean±SEM				
Microorganism	100	50	25	12.5	5 (Cipro)
E. coli	12.00±0.00^{a}	06.00±0.00^{b}	06.00±0.00^{b}	04.00±0.00^{c}	23.67±0.33^{d}
S. aureus	10.00±0.00^{a}	06.00±0.00^{b}	00.00±0.00^{c}	00.00±0.00^{c}	22.67±0.33^{d}
P. aeruginosa	12.00±0.00^{a}	04.00±0.00^{b}	00.00±0.00^{c}	00.00±0.00^{c}	19.67±0.33^{d}
B. subtilis	16.00±0.00^{a}	14.00±0.00^{b}	10.00±0.00^{c}	00.00±0.00^{d}	20.67±0.33^{e}
S. typhi	14.00±0.00^{a}	10.00±0.00^{b}	00.00±0.00^{c}	00.00±0.00^{c}	21.67±0.33^{d}

Values of mean across rows with different alphabetical superscripts are statistically different ($p<0.05$)

The dye extract showed antifungal activity against *P.notatum* (08.00±0.00^{a}) only at 100mg/mL, while there was no antifungal activity against *C. albicans* and *A.niger* at all concentrations as presented in Table 5. This suggest that the compounds in the dye extract may be selective against bacteria than fungi. Dye extract from *Swietenia macrophylla* was also reported to have no antifungal activity against *C. albicans* at all concentrations (Akhtar et al., 2022).

Table 5: Antifungal Activity of the Methanol Dye extract

	Extract concentration (mg/mL)/Zone of inhibition (mm)mean±SEM				
Microorganism	100	50	25	12.5	5 (Amphotericin B)
C. albicans	00.00±0.00^{a}	00.00±0.00^{a}	00.00±0.00^{a}	00.00±0.00^{a}	26.67±0.033^{b}
A. niger	00.00±0.00^{a}	00.00±0.00^{a}	00.00±0.00^{a}	00.00±0.00^{a}	25.67±0.033^{b}
P. notatum	08.00±0.00^{a}	00.00±0.00^{b}	00.00±0.00^{b}	00.00±0.00^{b}	21.67±0.033^{c}

Values of mean across rows with different alphabetical superscripts are statistically different ($p<0.05$)

Amphotericin (5mg/mL) was used as a positive control for the fungi. The dye and the control were both effective on the bacteria. Some dyes have been found to have strong antibacterial properties because they contain phenol, tannin, and quinone (Kanchana et al., 2013).

Minimum Inhibitory Concentration (MIC) of the Methanol Dye extract from Locust Bean Pod

The lowest concentration of the dye extract (anti-microbe) that can inhibit the growth of bacteria is known as the minimum inhibitory concentration (MIC). The MIC of the methanol dye extract of the locust bean pod is shown in Table 6. The minimum

concentration of the dye extract that inhibited E. coli and S. aureus was 12.5 mg/mL, B. subtilis and S. typhi was 25 mg/mL while P. aeruginosa and P. notatum was 50 mg/mL. Musa et al. 2018 also reported that the minimum inhibitory concentration (MIC) of the Hibiscus sabdariffa dye extract against Staphylococcus aureus was 12.5 mg/mL.

Table 6: Minimum Inhibitory Concentration (MIC) of the Methanol Dye extract

Microbes	100 mg/mL	50 mg/mL	25 mg/mL	12.5 mg/mL	6.25 mg/mL	MIC Value
E. coli	-	-	-	-	+	12.5
S. aureus	-	-	-	-	+	12.5
P. aeruginosa	-	-	+	+	+	50
B. subtilis	-	-	-	+	+	25
S. typhi	-	-	-	+	+	25
P. notatum	-	-	+	+	+	50

Keys: + =Growth in tube - =No growth in tube

Minimum Bactericidal Concentration (MBC) of the Methanol Dye extract from Locust Bean Pod

The lowest concentration of the dye extract that can completely kill the bacteria is known as the minimum bactericidal concentration, (MBC). The MBC of the methanol dye extract of the locust bean pod is shown in Table 7. The minimum concentration of the dye extract that killed E. coli, P. aeruginosa, S. typhi and P. notatum was greater than 100mg/ml while S. aureus and B. subtilis was 100 mg/mL.

Table 7: Minimum Bactericidal Concentration (MBC) of the Methanol Dye extract

Microbes	100 mg/mL	50 mg/mL	25 mg/mL	12.5 mg/mL	6.25 mg/mL	MBC Value
E.coli	+	+	+	+	+	>100
S.aureus	-	+	+	+	+	100
P.aeruginosa	+	+	+	+	+	>100
B.subtilis	-	+	+	+	+	100
S.typhi	+	+	+	+	+	>100
P.notatum	+	+	+	+	+	>100

Keys: + =Growth on plate - = No growth on the plate

Natural dyes have been shown in numerous studies to be effective at inhibiting microbial development in addition to imparting color. Because they include high concentrations of bioactive substances like tannins, flavonoids, and alkaloids that are effective against bacterial and fungal infections, several natural dyes offer exceptional antibacterial activity against microorganisms (Bhuyan 2016; Musinguzi et al., 2019). The

presence of tannins in the African locust bean pod dye extract, may likely be responsible for the antimicrobial properties. The ability of tannins to penetrate the bacterial cell wall and reach the interior membrane, where they disrupt the cell's metabolism and ultimately cause its death, contributes to their antibacterial efficacy (Kaczmarek, 2020).

UV/Vis Spectra of Dye Extract from Locust Bean Pod

The UV-visible spectra of the methanol dye extract of African locust bean pod, as represented on Figure 1 shows several distinct peaks in the absorbance spectrum. These peaks suggest the presence of various chemical compounds that absorb light at specific wavelengths.

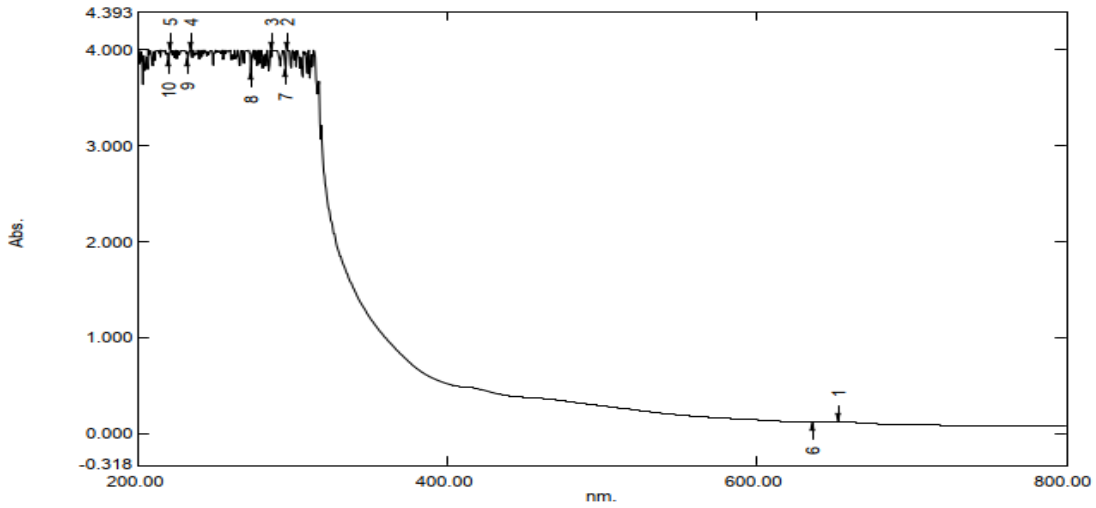

Figure 1: UV-Visible spectra of MLBP Dye extract

Peak at 287 nm is likely because of the carbonyl groups (C=O), which are characteristic of compounds like polyphenolic acids like hydrolysable tannins. This is because generally, tannins are absorbed in 204-284 nm and slightly high in the UV region (Purushotham *et al.,* 2024). Khatun and Mostafa 2022 reported a sharp peak at 230 nm and another at 245 nm, indicating the presence of different tannin components, which are ascribed to π-π* of the carbonyl group (Bukhari *et al.*, 2017; Zhou *et al.*, 2020). The Phenolic acids and flavonoids found in MLBP dye extract are particularly noteworthy as these compounds were known for their health benefits. Flavonoid, in particular, is known to have anti-cancer, antioxidant and anti-inflammatory properties (Akhtar et al., 2022). These compounds are commonly found in plant extracts and have antioxidant properties, and peak at 287 nm is also indicative of carbon-oxygen double while peak at 234.5 nm, could be attributed to aromatic amino acids or peptides, which are often found in plant

proteins. Peak at 221.5 nm is associated with aromatic rings, which are present in various compounds like alkaloids and terpenoids while in the visible region at 653 nm and 637 nm of the spectrum, could be due to the presence of compounds like carotenoids or anthocyanins, these also agree with the finding of Zhang et al. (2014) for profile of phytochemicals and antioxidant activities of extracts gotten from different solvents in cumin seeds. These compounds contribute to the color of plant extracts and can also have antioxidant properties which are consistent with previous studies on African locust bean extracts. Numerous biologically active substances, including flavonoids, phenolic acids, alkaloids, and terpenoids, have been found in studies. These compounds have been shown to possess antibacterial, anti-inflammatory, and antioxidant properties (Tuah *et al.,* 2017).

FTIR Spectra of Dye extracts from Locust Bean Pod

The FTIR spectra results of MLBP dye extract is presented in Figure 5. The peaks in 3000-2800 cm^{-1} region indicate the presence of C-H stretching vibrations. This suggest that aliphatic hydrocarbons (single-bonded carbon) in the dye.

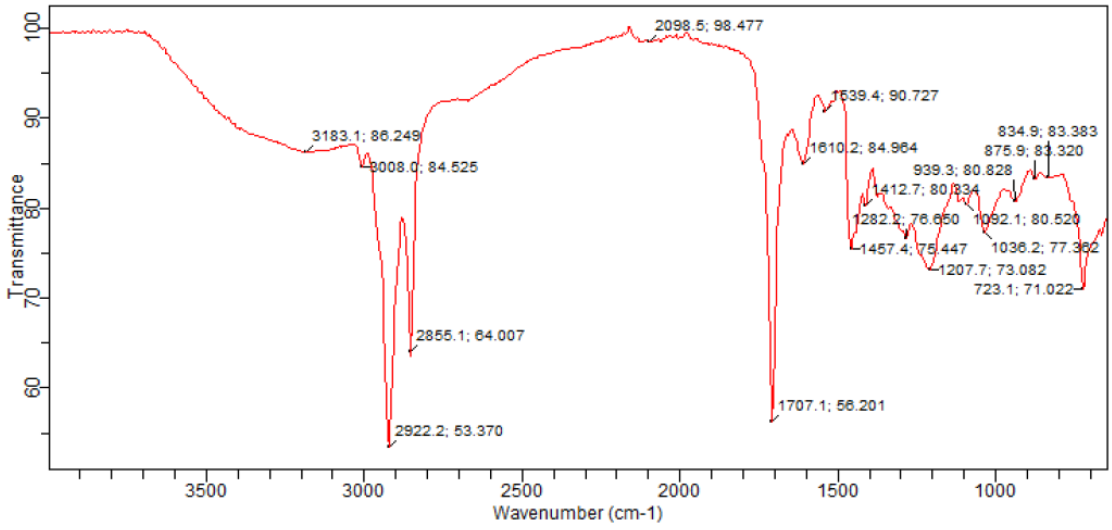

Figure 2: FTIR Spectra of the locust bean pod Dye extract

This peak 2098.5 cm^{-1} is typically associated with C≡C stretching vibrations, indicating the presence of a triple bond, possibly within an alkyne functional group. However, this peak might also be due to impurities in the sample. This peak 1707.1 cm^{-1} is likely due to C=O stretching vibrations, suggesting carbonyl groups. This could be due to carboxylic acids, esters, amides or ketones in the dye. These peaks in the 1600-1500 cm^{-1} region are associated C=C stretching vibrations in aromatic rings (benzene rings). This indicates the presence of aromatic compounds in the extract. These peaks 1457.4 cm^{-1} and

1412.7 cm^{-1} are attributed to C-H bending vibrations in aliphatic and aromatic hydrocarbons, further confirming their presence in the extract. These peaks 1282.2 cm^{-1}, 1207.7 cm^{-1} may be associated with C-O stretching vibrations in esters or carboxylic acids. However, without additional information, it's difficult to definitively assign these peaks. These peaks 1092.1 cm^{-1}, 1036.2 cm^{-1} are typically associated with C-O stretching vibrations I alcohols or esters. This suggest that these functional groups are in the dye. These peaks in 939.3 cm^{-1} -723.1 cm^{-1} region are indicative of various bending vibrations in aromatic rings and aliphatic hydrocarbons (Silverstein and Webster 2014). The Methanol dye of the African locust bean pod appears to contain a complex mixture of compounds with many functional groups. The presence of aliphatic hydrocarbons, aromatic compounds, carbonyl groups, alcohols and possibly esters suggest that the extract has diverse chemical composition (Boas and Castro 2022).

GC/MS Results of the Locust Bean Pod Dye extract

The GCMS results of the locust bean pod dye extracts with different peak areas and retention time attributed to various compounds is shown in Table 8:

Table 8: Compounds Identified from GC-MS of MLBP Dye extract

RT	Compound Detected	Molecular Formula	Molecular Weight	Peak Area%
4.58	1,3- Benzodioxol- 5-ol	$C_7H_6O_3$	138	0.59
5.90	Phenol, 2- methyl 5-(1-methylethyl)-	$C_{10}H_{14}O$	150	3.20
8.99	3-Allyl-6- methoxyphenol	$C_{10}H_{12}O_2$	164	0.65
9.56	n- Hexadecanoic acid	$C_{16}H_{32}O_2$	256	7.93
10.50	1,3- Benzodioxole	$C_7H_6O_2$	122	2.56
14.46	Tetra decanoic acid	$C_{14}H_{28}O_2$	228	1.51
15.10	1,8,11- Heptadecatriene	$C_{17}H_{30}$	234	2.28
17.30	9,12- Octadecadienoic acid	$C_{38}H_{68}O_8$	242	6.85
20.00	Vanillin	$C_8H_8O_3$	152	6.71
22.00	Tryptophan	$C_{11}H_{12}N_2O_2$	204	3.88
28.02	Octadecanoic acid	$C_{18}H_{36}O_2$	284	7.31
33.90	Methylparaben	$C_8H_8O_3$	152	4.15
34.00	Heneicosanoic acid	$C_{21}H_{42}O_2$	326	8.59
36.50	Campesterol	$C_{28}H_{48}O$	400	6.22
40.98	Lineoleoyl chloride	$C_{18}H_{31}ClO$	298	6.54
43.97	Oleoyl chloride	$C_{18}H_{33}ClO$	300	4.48
48.00	cis-9- Hexadecenal	$C_{16}H_{30}O$	238	14.81
49.96	Hexadecanoic acid, 2-	$C_{19}H_{38}O_4$	330	4.79

RT	Compound Detected	Molecular Formula	Molecular Weight	Peak Area%
	hydroxy-1- (hydroxymethy l)ethyl ester			
53.04	(+)-Sesamin	$C_{20}H_{18}O_6$	354	6.93

Table 8 showed a total of 19 compounds present in the methanol dye extract. Based on abundance, the major compound identified was cis-9-Hexadecenal (14.81%), followed by Heneicosanoic acid (8.59%), Hexadecanoic acid (7.93%), Octadecanoic acid (7.31%), (+)-Sesamin (6.93%), 9,12- Octadecadienoic acid (6.85%), Vanillin (6.71%) and Hexadecanoic acid, 2- hydroxy-1- (hydroxymethy l) ethyl ester (4.79%). Other prevalent compounds include: Phenolic compounds such as 1,3-Benzodioxol-5-ol ($C_7H_6O_3$) with peak area at 0.59%, Phenol, 2-methyl-5-(1-methylethyl)- ($C_{10}H_{14}O$) at 3.20%, 3-allyl-6-methoxyphenol, vanillin; Sterols in form of Campesterol ($C_{28}H_{48}O$) at 6.22% (are plant-based compounds that can have cholesterol-lowering effects. Campesterol is a common sterol found in plants) and Fatty acids: n-Hexadecanoic acid, tetradecanoic acid, 9,12-Octadecadienoic acid, Octadecanoic acid, Heneicosanoic acid which serve as energy sources and may also have bioactive properties. Babatunde, (2017) also reported a similar result of Hexadecanoic acid ethyl ester (19.02%), Octadecanoic acid ethyl ester (9.41%), ethyl paraben (9.15%), and Octadeca-9-enoic acid (7.86%) to be present in the dye of sorghum bicolour leaf. The extract's sterols, fatty acids, and phenolic components are in line with earlier research on African locust beans. These substances are said to have a variety of biological properties, including antibacterial, anti-inflammatory, and antioxidant properties. Aldehydes, including cis-9-Hexadecenal, were found at 14.81%, along with additional substances such oleoyl chloride, hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester, and (+)-Sesamin. The discovery of these substances raises the possibility that the methanol extract of African locust beans, which has anti-inflammatory, cholesterol-lowering, and antioxidant qualities, may offer health advantages.

HPLC Analysis of the Locust Bean Pod Dye extract

A total of fifteen (15) compounds were separated and identified from the HPLC results of the methanol dye extract. Figure 3 showed that gallic acid was identified as the most abundant. Other compounds identified were protocatechuic acid, catechin, o-hydoxybenzoic acid, chlorogenic acid, vanillic acid, syringic acid, caffeic acid, m-hydrobenzoic acid, p-coumaric acid, sinapic acid, t-ferulic acid, t-cinnamic acid, biochanin

A and anthrol. In general, there are two types of tannins: hydrolyzable tannins (pyrogallol) and condensed tannins (proanthocyanidins). The hydrolyzable tannins are made up of polyesters with a sugar moiety and organic acids called gallotannis and ellagitannins, which hydrolyze to produce gallic acid and ellagic acid, respectively (Shabbir et al., 2016). Tannin yields a range of colors, such as yellow, brown, gray, and black in natural dyes (Yusuf et al., 2017). Tannin is confirmed to be responsible for the brown color of the African locust bean pod dye extract.

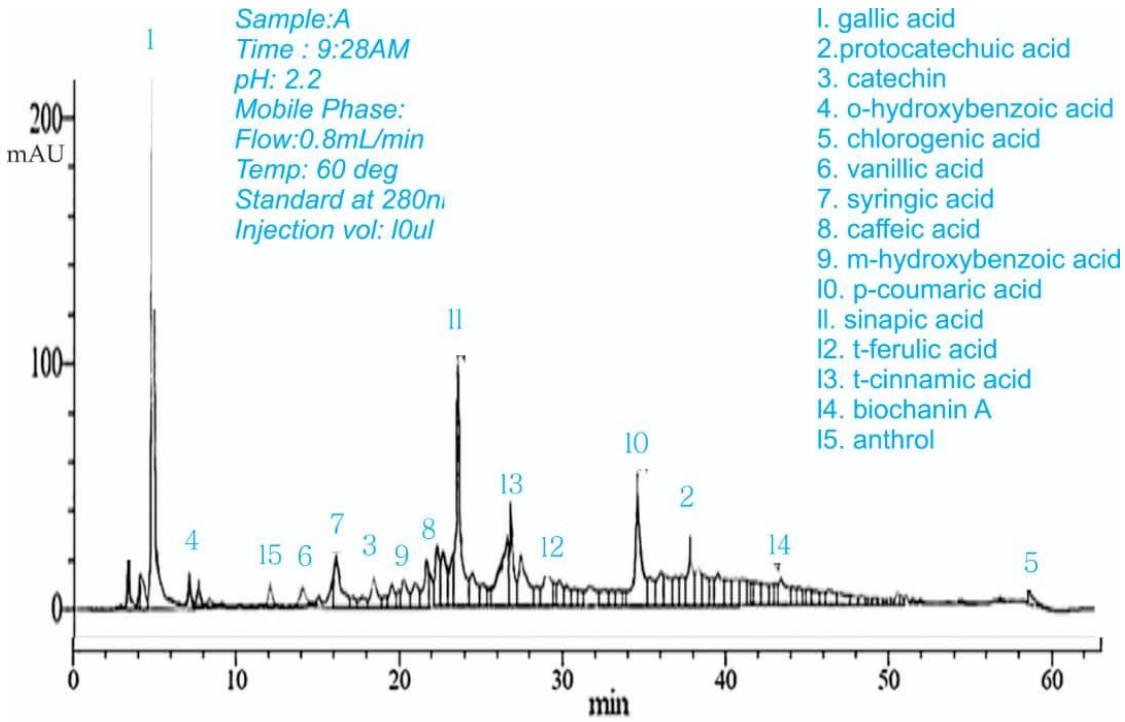

Figure 3: HPLC results of the Locust Bean Pod Dye extract

SEM Results of the Locust Bean Pod Dye extract

The SEM result of the locust bean pod methanol dye extract is presented in Plate 3. It reveals a highly irregular, rough and porous surface morphology. The microstructure is characterized by agglomerated particles with heterogeneous size distributed and visible micro-voids. The bright grey regions represent compacted, fused-like solid structure, while the darker areas indicate pores or void spaces (Auta and Hameed, 2011). The observed morphology indicates good absorption potential due to high surface roughness which enhances dye uptake efficiency.

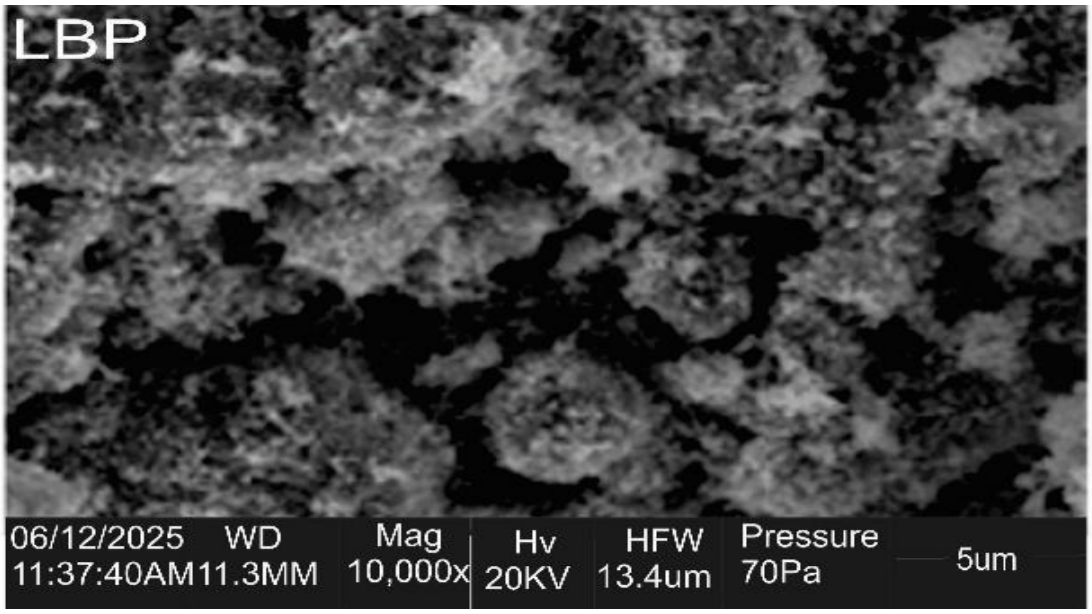

Plate 2: SEM Results of the Locust Bean Pod Dye Extract

CONCLUSION

The research concludes that *pakia biglobosa* dye extract obtained using maceration extraction demonstrated good extraction yields and stability. The physical properties of the dye extracts were evaluated to assess their suitability for textile and industrial applications. The results showed that the dye extract possessed distinct and stable physical characteristics. Phytochemical screening revealed the presence of key active compounds such as tannins, quinones, terpenoids, alkaloids, glyscosides, flavonoids, anthraquinones, steroids, anthocyanins which are responsible for coloration as well as added functional properties. The dye extract possesses notable antimicrobial activity, indicating that fabrics dyed with these natural dyes could offer added hygienic benefits. UV-Visible, FTIR, GCMS, HPLC and SEM analyses confirmed the complex chemical nature of the extract, showing functional groups associated with chromophores and adequate thermal stability suitable for textile processing. The findings of this research demonstrate that dye extract from *parkia biglobosa* is an effective natural dye for cotton fabric.

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