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Extraction and Characterization of Dye Extract from *Bridelia ferruginea*

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Abstract

This study investigated the extraction and characterization of a natural dye from the stem of *Bridelia ferruginea* using the Soxhlet method. The extraction yielded 32% dye extract, which exhibited a maroon color, a pH of 5.10, a melting point of 200–210 °C, a density of 1.20, partial solubility in water, and complete solubility in methanol. Phytochemical screening revealed the presence of alkaloids, glycosides, flavonoids, terpenoids, saponins, tannins, and steroids, whereas phenols and anthraquinones were absent. The dye extract also demonstrated inhibitory activity against certain bacterial and fungal organisms. Characterization using FTIR, UV-Visible spectroscopy, SEM, TGA, and GC-MS further elucidated its properties. FTIR analysis indicated the presence of O–H stretching, C–H stretching, C=C stretching, C–H bending, and C–O stretching functional groups. The UV-Visible spectrum showed distinct absorption peaks, with the most prominent peak observed at 304 nm and an absorbance value of 2.4889. SEM analysis revealed compact, fused, or crystalline-like surface structures. TGA showed thermal stability in three stages, with initial weight loss occurring between 30 and 150 °C, major weight loss between 250 and 450 °C, and residual mass remaining above 500 °C. GC-MS

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analysis identified 18 compounds with different retention times and intensities, among which maltose showed the highest intensity (2.50), while propanoic acid and tetral glycol showed lower intensities (0.20). These findings demonstrate that the stem extract of *Bridelia ferruginea* possesses notable physicochemical, phytochemical, antimicrobial, and structural characteristics relevant to its potential use as a natural dye.

Keywords: *Bridelia ferruginea*; Natural Dye; Soxhlet Extraction; Phytochemical Screening; Characterization

INTRODUCTION

A meaningful glimpse into our reality is provided by color. They provide the most appealing appearance to commonplace items like paper, plastics, paints, textiles, and food.. According to Kumar et al. (2021) A colorful substance with an affinity for the substrate it is applied on is called a dye. People are aware of the environmental effects of synthetic dyes because of the significant health dangers linked to their use in the textile sector. Consequently, the textile industry has shifted from using synthetic colors to using natural ones, particularly those derived from plants. (Thadepalli & Roy, 2022).

Natural dyes are substances derived from plants and animals that give food, cosmetics, medications, hair, fur, and polymers color. According to Osabohien (2009), Various items are dyed to enhance their appearance and attract buyers. Natural dyes can be derived from botanical components such as flowers, roots, leaves, bark, insect excretions, and minerals. Although dyes are abundant in nature, the processes used to make them can also give them varied shapes and uses. (Nwonye & Ezema, 2017).

To improve their appearance and draw in customers, a variety of things are dyed. Plant parts like flowers, roots, leaves, bark, insect secretions, and minerals can all be used to create natural dyes. Even while dyes are found in large quantities in nature, their manufacturing procedures can provide them a variety of forms and applications. (Thakker & Sun, 2021). The demand for natural dyes is rising since they are less dangerous than their synthetic counterparts. Synthetic colors are dangerous, carcinogenic, and possibly lethal. It requires an extensive duration to decompose when released into the natural environment. (Ismail et al., 2019).

Ayele et al. (2020) indicated that dyes obtain from natural sources have high degree of color fastness and can provide a diverse array of visually appealing hues. As a result, research is being done worldwide on the application of plant dye in the textile sector and the resurgence of natural dye as a substantial alternative to synthetic dyes. (Slama et al., 2021).

There are thousands of plants and animals which contain colourants that can be used as natural dyes. Bridelia ferruginea is a prominent shrub in the Euphorbiaceae family, utilized in traditional medicine to treat a number of ailments, such as arrow poisoning, bites, thrush, intestinal and bladder problems, skin diseases, chronic diabetes, arthritis, diarrhea, constipation, and oral infections. (Yeboah et al., 2022). This present study is therefore focused on the extraction, physical properties, antimicrobial, phytochemical screnning and characterization of the dye extract from Bridelia ferruginea.



Plate 1: Stem of Bridelia ferruginea

MATERIALS AND METHODS

Sampling and Sample Preparation

The samples of *Bridelia ferruginea* and *Pterocarpus erinaceus* was gathered from Noma village, Wukari Local Government, Taraba State. Before being extracted, the sample was dried at room temperature and pulverized with a metalic mortar and pestle into a powder.

Sohxlet Extraction

The analytical weighing balance was used to measure the sample. 200 g of the sample was divided into four parts, each of which contain 50 g of the sample. For each of the two 50 g samples that are fitted into the thimble for extraction, 200 mL of organic solvent was poured to the round-bottom flask. Each sample undergo an extraction process that lasted six hours. The solvent was removed using a rotary evaporator, leaving the dye to dry. (Rather et al., 2020).

Physical Properties of the Dye Extracts

Determination of pH of the Dye Extract

Standard buffer solutions with pH values of 4, 7, and 10 are used to calibrate the pH meter. A known quantity of dye was dissolved in distilled water to create the dye extract solution. After the dye solution stabilizes, the pH electrode was submerged in it and the pH value was recorded (ASTM D1293-12).

Determination of Melting Point of the Dye Extract

A melting equipment was used to determine the dye extract's melting point. After a narrow capillary tube has been closed at one end, a small amount of dye extract was added and heated gradually from room temperature until it melts. The melting point was register (ASTM E324).

Determination of Density of the Dye Extract

The empty pycnometer was weighed. The pycnomete was fill with the dye solution and weighed it again. Calculate the density using the formula. Density=Volume of solution/Mass of solution (ASTM D4052).

Solubility Test of the Dye Extract

The maximum amount of dye that may dissolve in a solvent at a given temperature is used to calculate solubility: A known volume of solvent was mixed with excess dye in a container. Until equilibrium is achieved, the mixture was stirred and kept at a consistent temperature. To get rid of the dye that hasn't dissolved, the solution was filtered. Gravimetric or spectrophotometric analysis was used to determine the dissolved dye's concentration. (ASTM E1148-02).

Phytochemical Screening of the Dye Extracts

The sample was subjected to phytochemical analysis using standard procedure as describe by

(Balamurugan et al., 2019).

Test for Anthraquinones

Five milliliters of extract were mixed with a couple milliliters of concentrated H_2SO_4 and one milliliter of diluted ammonia. Anthraquinones are confirmed to be present by the appearance of rose pink.

Test for Alkaloids

two drops of the reagent to a few milliliters of filtrate.A creamy or white precipitate indicates a positive result for alkaloids in the Mayer's test, which involves adding

Test for Glycosides

In the Borntrager's test, 3 milliliters of chloroform were added to 2 milliliters of filtrate and shaken. 2 ml of 10% ammonia solution was added after the chloroform layer was removed. The presence of glycosides is indicated by the pink color.

Test for Phenol

Gelatine test: Two milliliters of a 1% gelatine solution containing 10% $NaCl$ were added to five milliliters of extract.The presence of phenol is indicated by the appearance of a white precipitate.

Test for Tannins

A few drops of a neutral 5% ferric chloride solution were added to 5 milliliters of extract; the formation of a dark green color shows the presence of tannins.

Test for Flavonoids

10% lead acetate was added to 1 ml of extract. The yellow precipitate observed, indicate that the flavonoids are present.

Test for saponins

A 5 ml of distilled water were used to aggressively agitate 0.5 milligrams of extract. For saponins, the foaming creation observed indicate the presence of saponins.

Test for steroids

The presence of steroids is indicated by the emergence of red color and yellowish green fluorescence when 2 milliliters of extract, 2 milliliters of chloroform, and 2 milliliters of

concentrated H_2SO_4 are added.

Test for Terpenoids

After adding 1.5 ml of concentrated H_2SO_4 and 1 ml of chloroform along the tube's sides to 3 ml of theextract, the interface's reddish brown color is seen as a sign that terpenoids are present.

Determinal of Antimicrobial Screening of the Dye Extracts

Bridelia ferruginea stem bark extracts undergo first antimicrobial testing on the strains in question utilizing the hole-in-plate disc diffusion technique, as outlined by (Usman et al., 2018). A 6 mm cork borer was used to bore wells in the media, and 0.2 mL aliquots of the extracts in different concentrations (100 mg/mL, 50 mg/mL, and 25 mg/mL, or 20, 10, and 5 mg/hole, respectively) was placed inside the wells. After that, the agar plates was maintained for 24 hours at 37 ^oCin an incubator. Using a transparent meter rule, the widths of the inhibition zones for each extract was measured in millimeters following incubation. Three separate tests were conducted on each extract.

Characterization of Dye Extract

FTIR

A diamond crystal of ATR (Pike, Gladi ATR) was immediately exposed to an FTIR (Varian FT-IR Spectrometer 660) analytical sample of each dye, and the resulting spectra was adjusted for background air absorbance. A Varian Resolutions Pro was used to record the spectra, and samples was measured between 4000 and 400 cm-1(Bartošová et al., 2017).

UV-VIS Spectroscopy

The UV Vis spectrophotometer was used to analyze the extracts. After ten minutes of centrifuging at 3000 rpm, the extracts were filtered. The Shimazdu Spectrophotometer was used to scan the extracts in the wavelength range of 200–800 nm. The absorbance of

the prominent distinctive peaks was measured and recorded. Every analysis was conducted twice in order to confirm the spectrum (Raj 2016).

Scanning Electron Microscope (SEM)

The size and morphology of samples at different stages was investigated using a Quanta 250, FEI, Netherlands Scanning Electron Microscope. The samples were mounted on aluminum stubs with conductive carbon tape and sputtered with gold using a plasma sputtering apparatus under vacuum at 20 mA for two minutes. The samples will then be observed and imaged (Li et al., 2024).

Thermogravimetric Analysis (TGA)

The Shimadzu TGA-50H thermogravimetric analyzer model was used to examine the dye extract's thermal characteristics (El-Zaher et al., 2021).

Gas Chromatography-Mass Spectrometry (GC-MS)

Agilent Tech's GC 7890B, MSD 5977A, and mass detector were used for the GC–MS analysis. 1 L of the sample's supernatant was injected into the GC, and helium was used as the carrier gas at a flow rate of 1 ml/min. The temperature of the GC oven was set to start at $80^{o}C$, climb by $15^{o}C/min$, reach $200^{o}C$, then increase by $5^{o}C/min$ to $280^{o}C$, culminating in a 5-min isothermal at $280^{o}C$. The ionization voltage was set at 70 eV, and the ion source was set at $230^{0}C$(Ibrahim et al., 2021).

RESULTS AND DISCUSION

Extraction of dye from Bridelia ferruginea

Table1: Extraction of dye from *Bridelia ferruginea*

Extraction method	Sample	Weight of sample before ex-traction(g)	Weight of sample after extraction (g)	Amount of Dye extracted (g)	Percentage yield of Dye extract (%)
Sohxlet	Bridelia ferruginea	200	136	64	32

Table 1 shows the extraction of dye from *Bridelia ferruginea* via sohxlet method of extraction. 200 g each of the sample was use to carry out this extraction. The percentage yield obtained was 32%. The percentage yield obtained can be attributed to the high temperature which increase the kinetic energy in the solvent, thereby increasing the

solubility and diffusion of compounds leading to more efficient extraction. This claim was supported according to Azwanida (2015) in a review on the extraction methods use in medicinal plants, principle, strength and limitation. According to Sutrisna et al. (2020) natural dye extracted from mango leaf and sappan barks via maceration, sohxlet and reflux method of extraction also shows that sohxlet method of extraction have the highest percentage yield.

Physical Properties of Dye Extract From Bridelia ferruginea

Table 2: Physical properties of dye extract from Bridelia ferruginea

Physical properties	SDEFBF
pH	5.10
Melting point (^{o}C)	200-210
Density (g/mL)	1.2
Solubility(water)	Partially soluble
Solubility(methanol)	Soluble

SDEFBF= Sohxlet Dye Extract from *Bridelia ferruginea*

The Table 2. shows the physical properties of the dye extract obtained sohxlet method of extraction. The pH of the dye extracts is 5.10. The extract showed acidic. This may be as a result of more acidic functional groups like carboxylic acids and phenolic group are extracted via sohxlet method of extraction. The stability of the conjugate base of the extract may also contribute (Salauddin et al., 2021).

The melting point of the extracts is 200-210 for sohxlet method of extraction. The melting point of a substance depends on its chemical structure and purity. Sohxlet method of extraction involve prolong heating which could degrade some heat sensitive component in the dye. This degradation may alter the chemical structure (Salauddin et al., 2021).

The density of the dye extracts is 1.2 g/mL for sohxlet method of extraction. The density of the dye extract may be influenced by solvent type, dye concentration and extraction conditions. The density obtained via sohxlet method signifies that, small volume is require to achieve desired coloration (Egbujor et al., 2023)

The solubility of the dye extracts shows that both dye extracts obtained are partially soluble in water but soluble in methanol. Dye solubility determines whether a dye can be dissolved in water or other solvents to create a solution suitable for dyeing (Alegbe &

Uthman, 2024). Solubility depends on like dissolves like. Less polar solvent like methanol can dissolve compound that are partially polar and partially nonpolar.

Phytochemical Screening of Dye Extract From Bridelia ferruginea

Table 3. Phytochemical screening of dye extract from Bridelia ferruginea

Phytochemical con-Stituent	Bridelia ferruginea
Alkaloids	+
Glycocides	+
Flavonoids	+
Phenols	-
Terpenoids	+
Saponins	+
Tannins	+
Steroids	+
Anthraquinones	-

Key= + present, - Absent

The qualitative phytochemical investigation of dye extract from *Bridelia ferruginea* shown in Table 3. reveal that, alkaloid, glycocides, flavonoids, terpenoids, saponins, tannins and steroids are all present in Bridelia *ferruginea* dye extract. The presence of alkaloid indicating potential medicinal properties such as analgesic or antimalarial effects. Saponins and steroids may contribute to antimicrobial, anti-inflammatory and immune-boosting properties. The presence of tannins has astringent properties and can act as natural preservatives or antioxidants. Flavonoids indicate higher antioxidant potential, terpenoids have aromatic and therapeutic properties. Phenols and anthraquinones with antimicrobial and antioxidant activities are absent in *Bridelia ferruginea*. Mendili et al. (2025) reveal the phytochemical screening of natural textile dyes extracted from Tunisian lichens.

Antimicrobial Screening of Bridelia ferruginea

Table 4: Antimicrobial Screening of *Bridelia ferruginea*

Extract concentration (mg/mL)/Zone of inhibition (mm)Mean/SD

S/NMicroorganism 15		12.5	10	5	2.5	1.25
1 A. niger	20.3 ± 020	18.6 ±0.50	16.3 ± 0.50	14 ± 0.44	0.0 ± 0.00	0.0 ± 0.00
2 S aureus	23.7 ± 0.34	21.4 ± 0.45	18.7 ± 0.74	14.8 ± 0.82	9.6 ± 0.72	0.0 ± 0.00
3 P aeruginosa	22.6 ± 0.72	18.5 ± 0.36	16.4 ± 0.50	13.2 ± 1.50	8.3 ± 0.84	0.0 ± 0.00

S/NMicroorganism 15		12.5	10	5	2.5	1.25
4 A. claratus	20.5±0.40	19.7 ±0.54	17.8 ± 0.46	15.2±0.80	0.0 ± 0.00	0.0 ± 0.00
5 C. albicans	20.5±0.60	19.5±0.16	18.5 ±0.25	17.5±0.20	0.0 ±0.00	0.0 ± 0.00

The results shown in Table 4. Shows the significance of *Bridelia ferruginea* as a potential antimicrobial agent, showing varied effectiveness against the tested organisms. The zone of inhibition is observed at concentrations of 15, 12.5, 10, 5 and 2.5 mg/mL for S. aureus and P. aeruginosa with the highest inhibition at 15 mg/mL 23.7 ± 0.34 mm and 15 mg/mL 22.6 ± 0.72 mm respectively. While for A. niger, A. claratus and C. albicans with the zone of inhibition at 15, 12.5, 10, and 5 mg/mL with the highest inhibition at 15 mg/mL 20.3 ± 0.2 mm, 15 mg/mL 20.5 ± 0.40 mm and 15 mg/mL 20.5 ± 0.46 mm respectively. The observed inhibition for A. niger, suggests the extract is effective against certain fungi, which is valuable for antifungal applications. The inhibition in S. aureus is important because S. aureus is known for causing skin infections and other complications, and resistance to antibiotics is a growing concern. The inhibition of P. aeruginosa at higher concentrations is significant, given that this bacterium is notorious for its resistance to multiple antibiotics and association with hospital-acquired infections. It suggests the extract could be useful in developing treatments for such resistant bacterial strains. The inhibition against A. claratus highlights the potential antifungal properties of the extract. This could contribute to combating fungal pathogens in agriculture or medicine. The extract's effectiveness against C. albicans is notable because this fungus is a common cause of opportunistic infections, especially in immunocompromised individuals. The findings suggest the extract could support antifungal therapies for such cases. The concentration-dependent activity observed indicates that the efficacy of *Bridelia ferruginea* varies with dosage. The extract's broad-spectrum activity against both bacteria and fungi underscore its potential for developing natural antimicrobial agents in healthcare and agriculture. Gawish et al. (2017) reported that curcumin dye had the best antibacterial activity against bacteria and fungi.

FTIR Sohxlet Dye Extract From Bridelia ferruginea

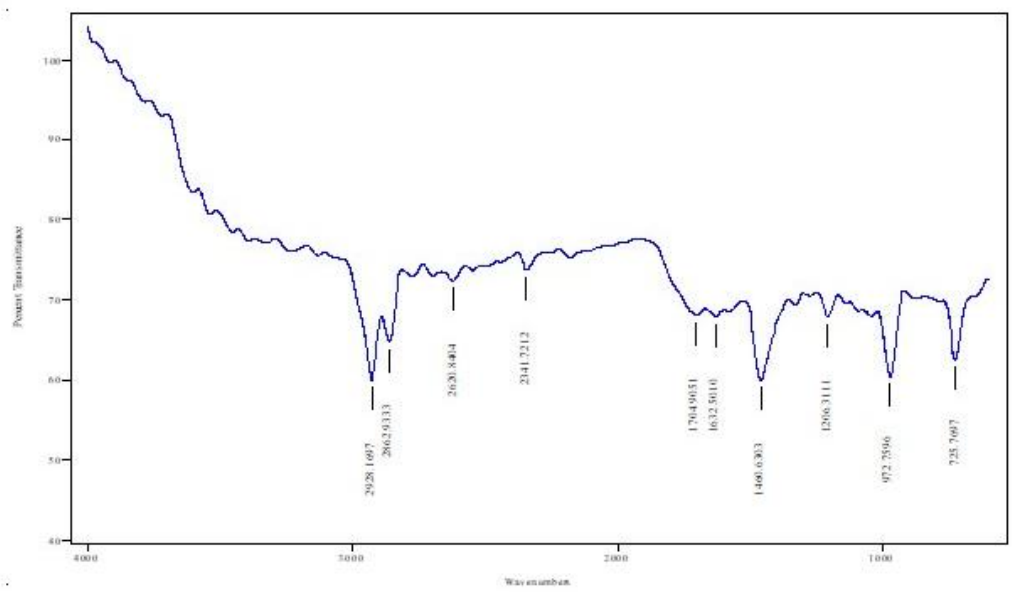

Figure 1: FTIR dye extract from Bridelia ferruginea

The spectra given in Fig 1. shows the FTIR result of dye extract obtained from Bridelia ferruginea via sohxlet extraction. At 2928.657 $cm-1$ and 2852.313 $cm-1$ these peaks are characteristicof$C-H$ stretchinginalkanes(asymmetricandsymmetric CH2 stretches). 2360.644 $cm-1$-2341.712$cm-1$ indicate the CO2 possible atmospheric interference. 1740.061$cm-1$ a strong peak in this region suggests a $C = O$ stretching, typically found in esters, aldehydes, ketones, or carboxylic acids. 1632.010$cm-1$ this may correspond to $C=C$ stretching in alkenes (Teweldebrihan et al., 2024). 1460.040 $cm-1$, often associated with $CH2$ bending (scissoring) vibrations in aliphatic hydrocarbons, 1382.111 $cm-1$ possibly related to C-H bending (deformation) of methyl groups $-CH3$. 972.596 $cm-1$ Could indicate out-of-plane bending of $= C - H$ in alkenes (Bekele et al., 2024). 721.697 $cm-1$, the region is commonly associated with rocking vibrations of $CH2$ in long-chain hydrocarbons.

UV-Visible of Sohxlet Dye Extract from Bridelia ferruginea

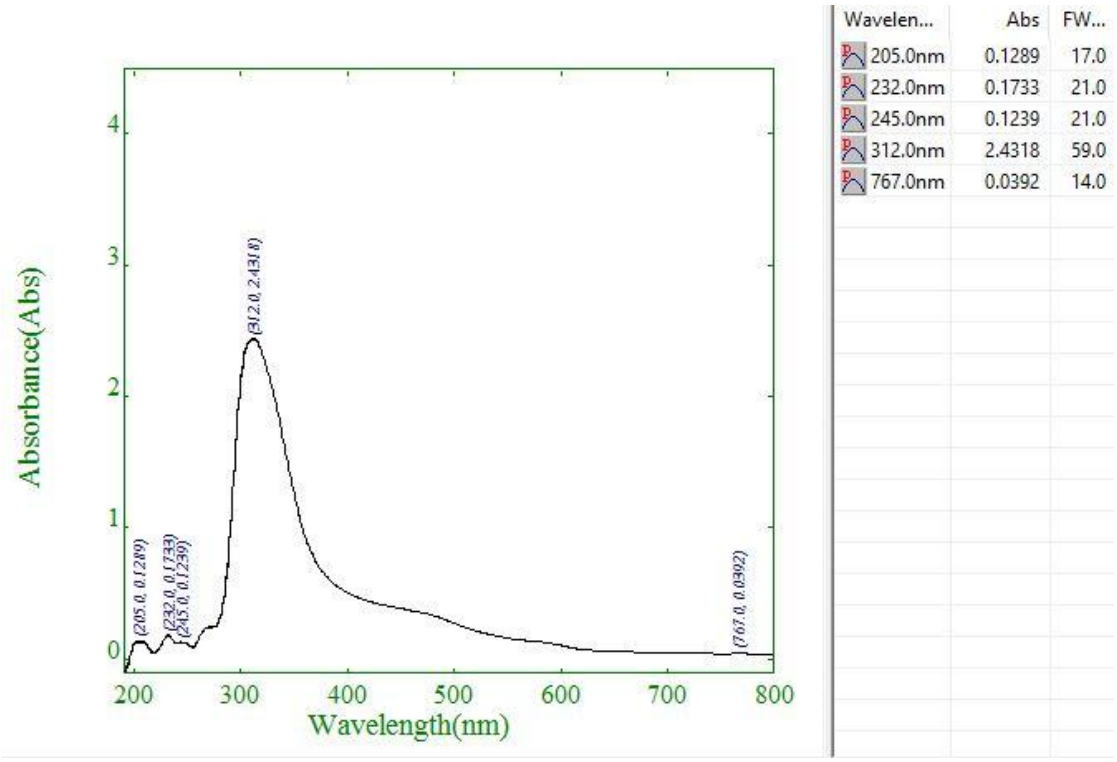

Figure 2: UV-Visible of dye extract from Bridelia ferruginea

The UV-Vis absorption spectrum provided highlights distinct peaks at specific wavelengths, with the most notable being at 312 nm with an absorbance value of 2.4318. Additionally, smaller peaks are observed at wavelengths such as 767 nm, 245 nm, 232 nm and 205 nm with the absorbance 0.0392, 0.1239, 0.1733 and 0.1289 respectively. Peaks at 205 nm, 232 nm, and 245 nm: These shorter wavelengths are typically associated with higher energy transitions, such as $n - \pi^*$ transitions in molecules containing lone pairs of electrons. Peak at 767 nm is in the near-infrared region and might suggest weak absorption due to overtone or combination bands. The absorbance peak at 312 nm is likely associated with chromophores that exhibit $\pi - \pi^*$ transitions. These chromophores are typically found in conjugated systems, such as aromatic rings or polyunsaturated compounds. Example include benzene derivatives, carbonyl groups and conjugated dienes. Leonarski et al. (2023) a review on enzymatic acylation as a promising opportunity to stabilizing anthocyanins absorbed in 240-400 nm in the UV region.

SEM for Sohxlet Dye Extract from Bridelia ferruginea

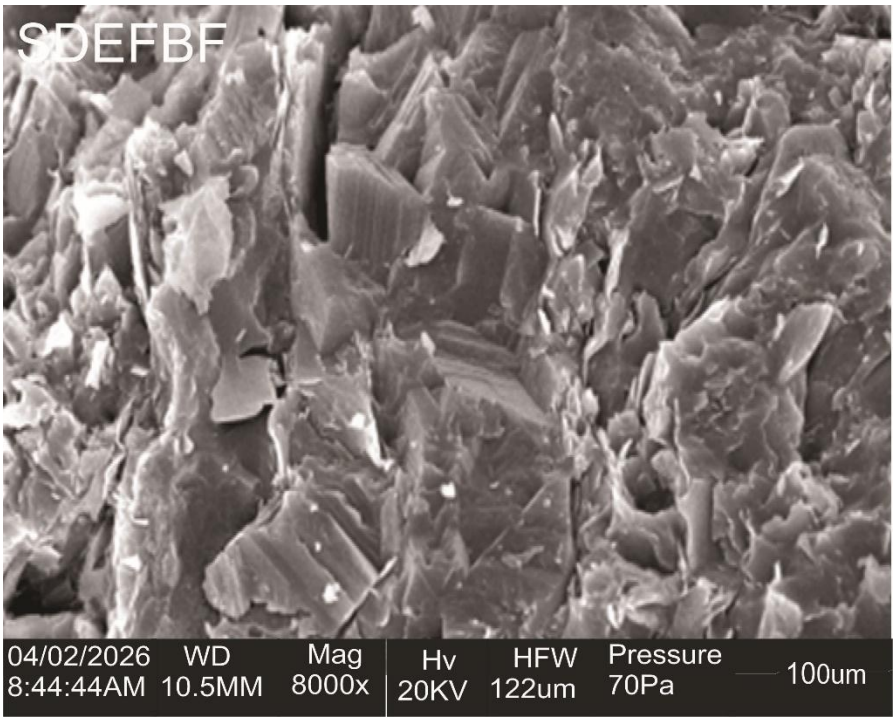

Figure 3: SEM for Sohxlet Dye Extract from Bridelia ferruginea

The Soxhlet dye extract from *Bridelia ferruginea* in Fig 3. exhibits more compact, fused, or crystalline-like structures. Soxhlet extraction involves prolonged heating, which enhances solubilization and extraction efficiency but may also promote partial structural modification or condensation of phenolic constituents. Denser morphology in Soxhlet extracts has been associated with higher extraction yield and concentration of high-molecular-weight compounds (Azwanida, 2015). The compact nature observed aligns with previous SEM studies of thermally extracted plant dyes, where particle agglomeration increased due to solvent evaporation and thermal concentration effects (Li et al., 2024). This morphology may influence dye fiber interaction by affecting solubility and penetration behavior during application.

TGA Sohxlet Dye Extract from Bridelia ferruginea

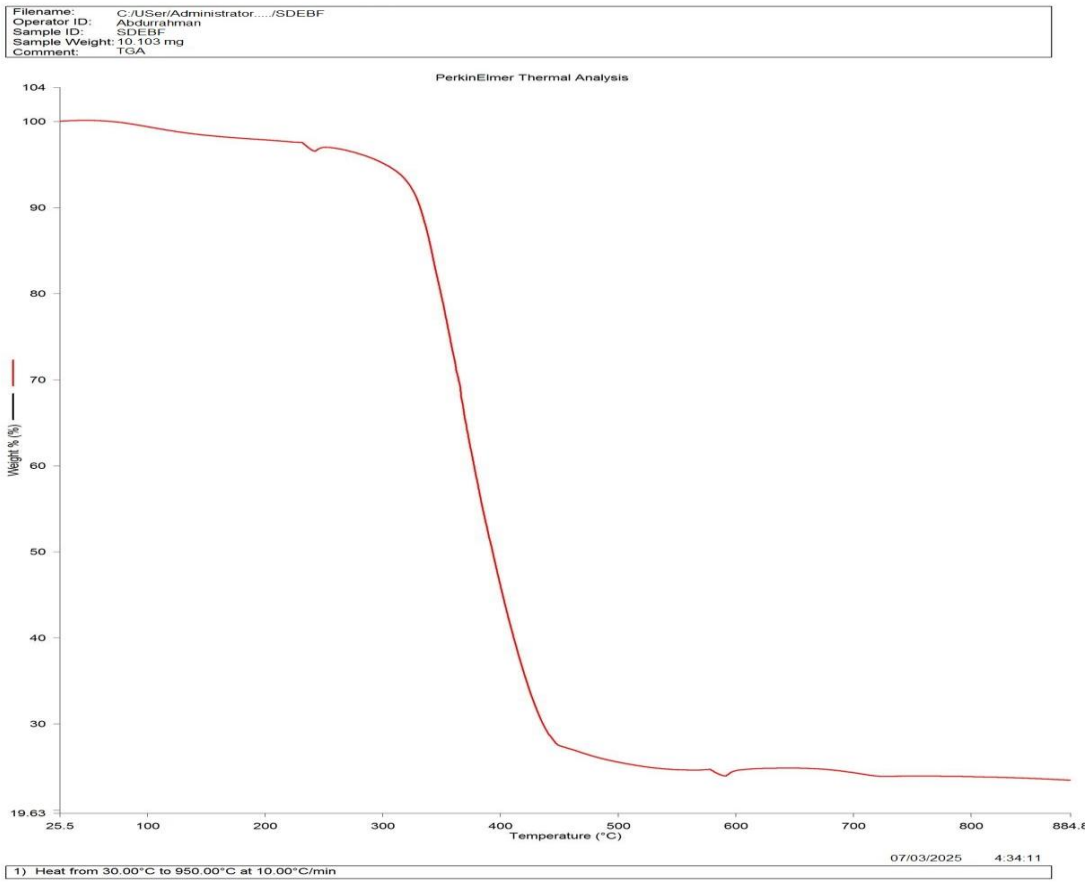

Figure 4: TGA dye extract from SDEFBF

The first weight loss was observed between 30 – 150 °C This weight loss is due to moisture desorption or loss of volatile additives. Similar low-temp mass losses are attributed to moisture or physically adsorbed solvents in bio-based polyesters and epoxy networks (ASTM E1131-20). The major weight loss is between 250-450 °C. This indicates thermal degradation of the polymer backbone particularly aromatic or ester-containing structures such as bisphenol-F epoxy or derivatives. Polymeric systems containing aromatic backbones such as bisphenol-F epoxy resins show major degradation between 300-450 °C due to scission of C–O and C–C bonds (Bekhouche et al., 2023). The residual mass is above 500 °C Signifies formation of thermally stable carbonaceous char, indicating good thermal stability and possibly flame-retardant behavior. High char yield at elevated temperatures is commonly observed in materials modified with phosphorus/nitrogen systems (Xie & Yang, 2024).

GCMS Dye Extract from Bridelia ferruginea

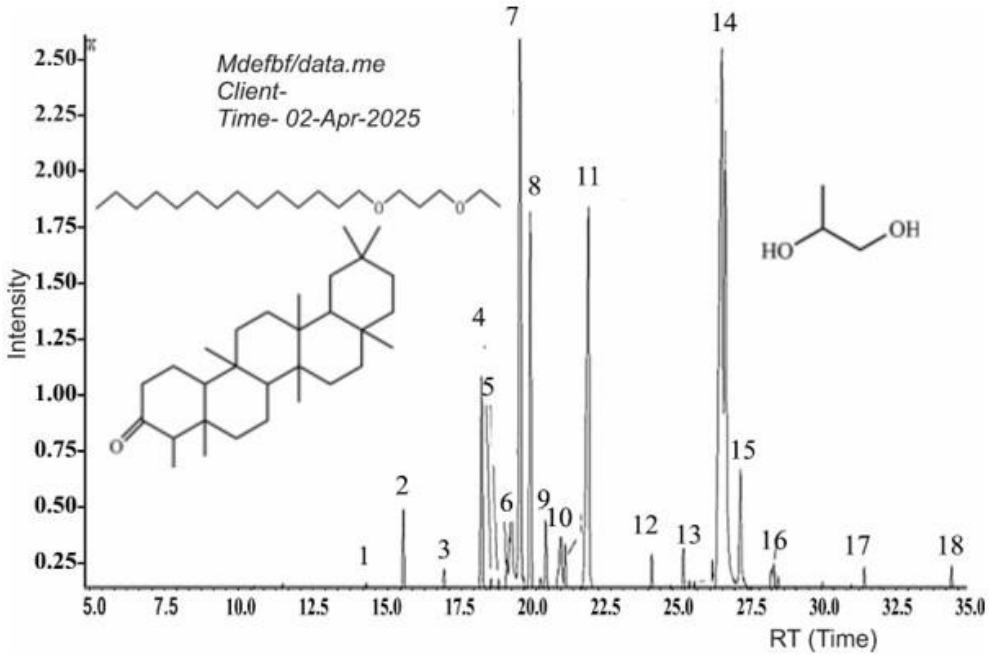

Figure 5: GCMS Dye Extract from MDEFBF

Fig 5. Shows the Gas Chromatography (GCMS) of dye extract. A total of 18 compounds were identified with their retention times (RT) in minutes, and respective intensities. The identify compound including organic acids (e.g., Propanoic acid, Dichloroacetic acid), sugars (e.g., d-Glucose, Trehalose, Maltose), and other compounds like Acetamide and Dodecane. The retention time indicates the time each compound took to pass through the GC column. For example, Propanoic acid had an RT of 14.30 minutes, while Uridine had the longest RT at 34.42 minutes. The intensity reflects the concentration of each compound. Dichloroacetic acid and Maltose showed the highest intensities at 2.50, indicating their significant presence in the sample. In contrast, compounds like Propanoic acid and Tetral gyvcol had much lower intensities of 0.20. The GCMS study of acetonic extract powder of date palm pits revealed the phenolic groups responsible for the coloring of the polyester fibers and confirmed the presence of certain compounds having antifungal, antibacterial, antioxidant, and anticancer activities (Souissi et al., 2024).

CONCLUSION

The research concludes that Bridelia ferruginea dye extract obtained using soxhlet 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, influenced by the extraction method and plant source. The dye extract possesses notable antimicrobial activity, indicating that fabrics dyed with these natural dyes could offer added hygienic benefits. Phytochemical screening revealed the presence of key dye-active compounds such as tannins, flavonoids, phenols, anthraquinones, and glycosides, which are responsible for coloration as well as added functional properties. FTIR, UV–Visible spectroscopy, SEM, TGA and GC–MS 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 extracts from Bridelia ferruginea are effective natural colourants for cotton fabric.

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