



APPLIED RESEARCH AND INNOVATION

ISSN: 2993-8988 (ONLINE)

VOLUME 2 ISSUE 2 (2024)

PUBLISHED BY
E-PALLI PUBLISHERS, DELAWARE, USA

Aqueous Extraction and Characterization of Eucalyptus Globulus for Use as Natural Dyes

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Article Information

Received: September 03, 2024

Accepted: October 11, 2024

Published: November 14, 2024

Keywords

Dyes, Eucalyptus, Fastness Ratings, Pre-Metallized Substrates, Spectral Characteristics

ABSTRACT

This study investigates the extraction, characterization, and application of natural dyes from Eucalyptus bark and leaf granules using solvents such as 2-ethoxy-ethanol, acetone, methanol, and distilled water. The dyes were characterized using Fourier Transform Infrared Spectroscopy (FTIR) and UV-visible spectroscopy with analyses performed using a Bruker Advanced 400 MHz FT spectrophotometer. FTIR analysis identified key functional groups such as hydroxyl (O-H), carbonyl (C=O), and aliphatic (C-H), indicating the presence of phenolic compounds, flavonoids, tannins, and fatty acids. Fastness tests, including wash, light, and rubbing fastness, were conducted and assessed on gray and blue scales, revealing differences in hue and shade between pre-metallized and un-pre-metallized substrates. Dyes extracted from leaves in distilled water and methanol showed higher absorbance, while bark extracts provided deeper brown shades on cotton fabrics. Leaf extracts produced lighter, greenish-brown shades. The fastness ratings ranged from very good to excellent, demonstrating the potential of Eucalyptus-based dyes for sustainable textile applications.

INTRODUCTION

A dye is a colored substance that forms a chemical bond with the substrate it is applied to, which sets it apart from pigments that do not chemically bind to the material. Dyes are typically used in aqueous solutions and may require a mordant to enhance their fastness on fibers (Ooth *et al.*, 2002). Most natural dyes are obtained from non-animal sources, such as roots, berries, bark, leaves, wood, fungi, and lichens (Lellis *et al.*, 2019). However, due to the high demand and advancements in technology, the majority of modern dyes are now synthetically produced from petrochemical sources. Some dyes are also derived from insects or minerals (Pereira *et al.*, 2021; Mahltig, 2024).

Synthetic dyes, created using various chemicals, are favored for their cost-effectiveness, superior optical properties, and durability (Booth, 2000). Both dyes and pigments are colored because they selectively absorb certain wavelengths of visible light. While dyes are generally soluble in solvents, pigments remain insoluble. Certain dyes can also be converted into lake pigments by adding salt to render them insoluble.

LITERATURE REVIEW

The practice of textile dyeing dates back to the Neolithic era. Throughout history, people have dyed their textiles using readily available local materials. Rare dyestuffs that produced vivid and lasting colors, such as the natural invertebrate dyes Tyrian purple and crimson kermes, were highly valued luxury items in ancient and medieval times. Plant-based dyes like woad, indigo, saffron, and madder played a significant role in the trade economies

of Asia and Europe. In Asia and Africa, resist dyeing techniques were commonly used to create patterned fabrics by controlling the absorption of color in piece-dyed textiles. New World dyes, such as cochineal and logwood, were introduced to Europe by Spanish treasure fleets (Ooth *et al.*, 2002). While European dyestuffs were carried to America by colonists (Balter, 2009). Dyed flax fibers have been discovered in a prehistoric cave in the Republic of Georgia, dating back to 36,000 BP (Balter, 2009; Kvavadze *et al.*, 2009). Archaeological findings indicate that dyeing practices have been prevalent for over 5,000 years, especially in India and Phoenicia. Early dyes were extracted from animal, vegetable, or mineral sources with minimal processing. The majority of dyes were derived from plant materials such as roots, berries, bark, leaves, and wood, though only a few of these are used commercially today.

The first synthetic dye, mauve, was accidentally discovered by William Henry Perkin in 1856 (Hubner, 2006; Travis, 1990). This breakthrough marked the beginning of a surge in synthetic dyes and advancements in organic chemistry. Subsequent aniline dyes, including fuchsine, safranin, and induline, were developed, and thousands of synthetic dyes have been created since then (Langhals, 2004). The discovery of mauve played a pivotal role in advancing fields like immunology and chemotherapy. In 1863, the precursor to Bayer AG was established in what is now Wuppertal, Germany. Later, in 1891, Paul Ehrlich observed that certain dyes were selectively absorbed by specific cells or organisms. He hypothesized that, if the dye did not harm other cells, a sufficiently high dose

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could be used to eliminate pathogenic microorganisms. Ehrlich applied this principle to treat syphilis, marking the first use of a chemical agent to selectively kill bacteria within the body. He also utilized methylene blue to target the plasmodium parasite responsible for malaria (Xu *et al.*, 2004).

Chemistry

The color of a dye depends on its ability to absorb light within the visible range of the electromagnetic spectrum (380-750 nm). According to the earlier Witt theory, a colored dye consisted of two components: a chromophore, responsible for color by absorbing light in the visible spectrum (e.g., nitro, azo, and quinoid groups), and an auxochrome, which enhances the color. However, this theory has been replaced by modern electronic structure theory, which explains that the color of dyes results from the excitation of valence π -electrons when exposed to visible light (Langhals, 2004; Benkhaya *et al.*, 2019).

Types of Dyes

Dyes are categorized based on their solubility and chemical properties (Ooth *et al.*, 2002), including acid dyes (water-soluble anionic dyes used for fibers like silk, wool, nylon, and some synthetic food colors), basic dyes (water-soluble cationic dyes for acrylic, wool, and silk), direct dyes (used on cotton, paper, leather, wool, silk, and nylon, and also serving as pH indicators and biological stains), laser dyes (used in lasers, optical media, and camera sensors), mordant dyes (requiring a mordant for enhanced fastness and commonly found among natural dyes), vat dyes (insoluble in water and requiring reduction in alkaline solutions for application, like indigo for denim), reactive dyes (featuring chromophores that form covalent bonds with fibers), disperse dyes (designed for dyeing cellulose acetate and polyester), azoic dyes (creating insoluble Azo dyes directly on or within fibers for vibrant, permanent colors), and sulfur dyes (inexpensive options for producing dark hues on cotton fabrics), with each type chosen based on its compatibility with fibers and desired properties, such as color intensity and fastness (Benkhaya *et al.*, 2020; Ferreira *et al.*, 2004). Nature offers a rich variety of plants that can yield colors for dyeing, many of which have been utilized since ancient times. In the early 21st century, the textile industry has witnessed a resurgence in the use of natural dyes. Western consumers have become increasingly concerned about the health and environmental effects of synthetic dyes, leading to a growing preference for products made with natural dyes. Capturing the market with naturally dyed fabrics is essential to ensure a safer environment. Traditionally, both natural and synthetic textile materials were dyed to enhance their value, appearance, and customer appeal. Initially, coloring textiles relied on natural sources until synthetic dyes were developed and commercialized. Due to their availability, purity, and cost advantages, synthetic dyes became the preferred choice

for most textile dyers and manufacturers (Olorunnisola & Oshifeso, 2016; Sivakumar *et al.*, 2009).

However, natural dyes, though known for lighter shades and lower colorfastness, can achieve better results with the help of mordants—chemicals that improve dye adherence. Mordants are metallic salts that create a bond between the fabric and dye, making them insoluble in water. The metal ions in mordants act as electron acceptors, forming coordination bonds with dye molecules. While chemical mordants are commonly used, natural mordants such as rice stalks and aluminum sulfate are also gaining prominence. Natural dyes can produce a diverse range of beautiful shades with acceptable levels of colorfastness, and they harmonize well together, making design easier. In contrast, synthetic dyes, although brighter, can appear harsh and often require greater skill for color matching (Rehman *et al.*, 2020; Ezenwobodo & Samuel, 2022).

Dyestuffs are categorized as fugitive and non-fugitive colors, derived from various sources. Although nearly all organic materials will produce some color when boiled in a dye bath, only certain plants yield colors that can function as dyes. Natural dyes can be obtained from multiple sources, including leaves, stems, twigs, flower heads, bark, roots, outer skins, hulls, husks, heartwood, wood shavings, berries, seeds, and lichens (Franca *et al.*, 2020; Garba & Abubakar, 2002).

Examples of plants that produce natural dyes include: Madder produces an orange to red dye, hollyhock yields a pink dye, hibiscus provides a reddish-purple dye, dandelion offers a yellow dye, indigo generates a navy blue dye, lavender results in a silvery gray or pale yellow dye and marigolds produce an orange-yellow dye (Ooth *et al.*, 2002).

MATERIALS AND METHODS

Eucalyptus granules were used to extract natural dyes in aqueous solutions using various polar solvents (ethanol, acetone, methanol, and distilled water). The extracted dyes were characterized using UV-visible and FTIR spectroscopy. These dyes were then applied to untreated and pre-metallized cotton fabrics, using aluminum sulfate and sodium chloride as mordants. The colorfastness of the dyed fabrics was evaluated using gray and blue scales.

Extraction

Eucalyptus bark and leaf granules were extracted using ethanol, sodium chloride, aluminum sulfate, and distilled water. The bark was extracted at room temperature for 24 hours, while the leaves were extracted using distilled water at boiling point for 1 hour. The extraction conditions for each solvent are summarized in Table 1.

Mordanting

A 20-gram sample of bleached cotton fabric was soaked in a solution containing 2% aluminum sulfate and 5% sodium chloride, with a liquor-to-fabric ratio of 20:1. The sample was boiled for one hour, then rinsed, washed thoroughly, and dried.

Dye application

Specific weights of Pre-metallized (mordanted) and unpremetallized cotton fabrics were immersed in a solution containing 400 ml/l of dye extract and dyed for about 15 mins. at a temperature of 45°C. 5% o.w. of sodium chloride was then added to the dyebath and dyeing continued at the boil for 45 mins. After dyeing, the fabrics were rinsed, washed thoroughly under running tap water and dried at room temperature.

Fastness Properties Test

Washing Fastness Test

The dyed fabric of size 4cm x 10cm was attached to a piece of the adjacent fabric containing (cotton and polyester), also measuring 4cm x 5cm, by sewing along one of the shorter sides with the fabric next to the face of the dyed fabric. The sewed specimen was placed in the wash bottle and the necessary amount of soap solution (5 g of premier soap and 2 g of sodium carbonate per litre) was added in a liquor ratio of 50:1 and the washing fastness is carried out in a warm water for 30 minutes. After that stipulated time, the sewed specimen was removed, rinsed twice in cold water, squeezed and dried. The change in colour of the specimen and the staining of the adjacent fabrics were assessed with grey scales.

Fastness to Rubbing

Dry rubbing

The natural dyed specimen was mounted to the holding clamp on the baseboard of the crock meter which measures 21 by 6 cm. A dry rubbing cloth was mounted flat over the end of the peg on the crock meter and fixed by means of the spring clip provided. The specimen was rubbed back and forth over a straight track 100 mm ± 8 mm long for 10 complete cycles (10 times back and forth) at one cycle per second. Staining of the rubbing cloth was assessed with grey scale.

Wet rubbing

A rubbing cloth was wet with distilled water and squeezed between blotting papers and the tests were carried out as the procedure for dry rubbing. The tested rubbing cloth was allowed to dry at room temperature. Staining of the rubbing cloth was assessed with grey scale (Uddin Banna *et al.*, 2019).

Fastness to light

The sample to be tested were exposed to light frame lamp with the same characteristics from the natural light but with a much higher intensity called the (Xenon Arc Lamp) light Fastness tester. The dyed samples were measured 10cm by 5cm (Ethanol, methanol and distilled water dyed samples) from the whole fabric. The samples were fastened to the holder then placed into the cell of the Fastness tester (Xenon Arc Lamp). The specimens were exposed to light and each test sample is partly coloured during the exposure. The samples were exposed for 48 hours. The change between the exposed and covered parts of the test sample (dyed samples) were then compared with the change between the covered and exposed area of the standard (blue scale) which shows the change in shades and the number represent the degree of fading of the dyed samples.

Ultraviolet/Visible Spectroscopy

2mls of the liquid dye extract was measured into a 30ml flask and diluted with 8mls of each of the solvent used for the extraction to the mark. The solution was vigorously shaken to ensure uniform dispersion of the dye in each solvent, absorbance was selected as the measurement mode for the run and its recording range was set at 1 to 4. After which the range of the wavelength scans was set 550nm and a sampling interval of 1nm. 1ml of the liquid dye was pipetted into the cuvette and the sample was run and the results were recorded and saved.

Fourier's Transform Infrared Spectroscopy

The Cary 630 FTIR, (Agilent Technology, USA), utilises advanced Fourier transform infrared (FTIR) spectroscopy technology, which is widely recognised as the most advanced method for identifying and quantifying molecular compounds. FTIR utilises an infrared light source to pass through the plasma exfoliated dye and onto a detector, which accurately measured the light absorption of the dye. Through the process of measuring absorbance, a distinct spectral fingerprint was generated. This fingerprint is then utilised to analyse the molecular structure of the dye and accurately quantify the amount of a specific compound within the mixture

RESULTS AND DISCUSSION

Table 1: Showing results for dye extracted from eucalyptus plant (bark and leave granules)

Plant (Eucalyptus)	Solvent	Weight of granules (g)	Volume of solvent (mls)	Volume of extract (mls)	Time (Hours)
Bark	Ethanol	200	2000	1080	24
Bark	Methanol	200	2000	860	24
Bark	Acetone	200	2000	1160	24
Bark	Distilled water	200	1600	1040	12
Leave	Ethanol	200	2000	1020	24
Leave	Acetone	200	2000	1120	
Leave	Methanol	200	2000	840	24
Leave	Distilled water	200	1600	10200	12

Table 1 presents the results of dye extraction experiments using eucalyptus bark and leaf granules, detailing the solvents, weights, volumes, extract yields, and extraction times. Ethanol consistently produced higher extract volumes than methanol, acetone, or distilled water, which was less efficient, especially in smaller volumes. The plant part (bark or leaf) may also influence extraction efficiency, but more data is needed for definitive conclusions.

The results presented in Table 1 indicate that the dyes extracted gave good yield (v/v) with higher volume from ethanol and distilled water perhaps due to their higher polarity. Extracts from eucalyptus bark gave rich brown coloration while those from the leaves appeared greenish

brown. Dyes extracted from acetone and ethanol showed deeper shades than those of the other solvents. The greater depth of shade arising from acetone and ethanol and water extracts could be attributed to their higher polarity compared to methanol. The dyed pre-metallized fabrics appeared deeper in shade than their un-pre-metallized counterparts. Reasons for this is thought to be that the mordants created more reactive sites in the cotton cellulose molecule which gave rise to more dye attraction and uptake consequently resulting to more dye molecules being attached to the cellulose polymer thereby giving deeper shades.

Table 2: Showing Results for Light Fastness Test of Bark and Leave Granules Dyes

Solvent	Mordanted fabric				Non-mordanted fabric			
	Bark		Leave		Bark		Leave	
	CSH	STN	CSH	STN	CSH	STN	CSH	STN
Ethanol	5	4-5	4-5	4	4-5	3-4	4	3
Methanol	4-5	4	4	4	4	3	3-4	3
Acetone	5	4-5	4-5	4-5	4-5	4	4	4
Distilled water	4-5	4-5	4	3-4	4	3	3-4	3

KEY: CSH = Change in Shade, STN = Staining of white

Extracted from Different Solvents on both mordanted and un-mordanted fabrics

The change in shade (CSH) indicates how much the shade of the dyed fabric changes when exposed to light, while staining of white (STN) represents the degree of staining on adjacent white fabrics when the dyed fabric is subjected to light; using ethanol as a solvent, mordanted fabrics showed excellent color stability and less staining (bark: CSH = 5, STN = 4-5; leaves: CSH = 4-5, STN = 4) compared to non-mordanted fabrics (bark: CSH = 4-5, STN = 3-4; leaves: CSH = 4, STN = 3), while with methanol, mordanted fabrics again showed better fastness properties (bark: CSH = 4-5, STN = 4; leaves: CSH = 4, STN = 4) than non-mordanted fabrics (bark: CSH = 4, STN = 3; leaves: CSH = 3-4, STN = 3); when acetone was used, both mordanted and non-

mordanted fabrics exhibited excellent fastness properties (mordanted bark: CSH = 5, STN = 4-5; leaves: CSH = 4-5, STN = 4-5; non-mordanted bark: CSH = 4-5, STN = 4; leaves: CSH = 4, STN = 4); using distilled water as a solvent, mordanted fabrics (bark: CSH = 4-5, STN = 4-5; leaves: CSH = 4, STN = 3-4) outperformed non-mordanted fabrics (bark: CSH = 4, STN = 3; leaves: CSH = 3-4, STN = 3), with bark-based dyes generally showing superior fastness properties compared to leaf-based dyes across all solvents used, and overall, mordanted fabrics consistently exhibited better light fastness and staining resistance than non-mordanted fabrics, with acetone being the best solvent for achieving higher light fastness, indicating that mordanting improves the binding of dyes to fabric, making them more resistant to light exposure and preventing dye leaching.

Table 3: Showing Results for Light Fastness Test of Bark and Leave Granules Dyes

Solvent	Mordanted		Non-mordanted	
	Bark	Leave	Bark	Leave
Ethanol	7	6	6	5
Methanol	6	6	5	4
Acetone	7	6	6	5
Distilled water	7	6	5	4

Extracted from Different Solventson both mordanted and un-mordanted fabrics

The light fastness test results reveal that mordanted fabrics generally exhibit superior resistance to light exposure compared to non-mordanted fabrics. For bark-based dyes, the mordanted samples scored consistently

high across all solvents, achieving a light fastness rating of 7, which indicates very good light stability. In contrast, the non-mordanted bark fabrics showed slightly lower scores, with 6 for ethanol and acetone, and 5 for methanol and distilled water, indicating a reduced resistance to light exposure. For leaf-based dyes, the

mordanted fabrics obtained a uniform score of 6 across all solvents, signifying good light fastness. However, the non-mordanted leaf fabrics demonstrated lower scores, ranging from 4 (moderate) when methanol or distilled water was used, to 5 (good) for ethanol and acetone as solvents. Mordanted fabrics consistently displayed better light fastness than non-mordanted fabrics, regardless of the dye source (bark or leaves) and the solvent used for

extraction. This suggests that mordanting enhances the stability of the dyes against light exposure. Additionally, bark-based dyes performed better than leaf-based dyes in both mordanted and non-mordanted conditions, indicating that bark may be a more effective source of light-resistant dyes.

Fastness to Rubbing

Table 4: Showing Results for Rubbing Fastness Test.

Dye	Granules	Un-mordanted dyed sample		Mordanted dyed sample	
			dry	wet	dry
Ethanol extract	Bark	3	4	4-5	4-5
	Leave	4	4-5	4	4
Methanol extract	Bark	3-4	4-5	4-5	4-5
	Leave	4	4-5	4-5	4-5
Acetone extract	Bark	4-5	4	4-5	4-5
	Leave	3	4-5	5	5
Distilled water extract	Bark	4-5	4-5	4	4
	Leaf	4-5	4-5	4-5	4-5

Mordanted fabrics consistently showed higher resistance to wet and dry rubbing compared to un-mordanted fabrics across all dye sources and solvents, with ethanol-extracted bark-based dyes demonstrating moderate resistance in wet (scoring 3) and good in dry (scoring 4) for un-mordanted samples, and improved resistance for mordanted samples (scoring 4-5 in both conditions), while leaf-based dyes scored similarly for un-mordanted samples (4 in wet, 4-5 in dry) and maintained consistent scores for mordanted samples (4 in both conditions); methanol-extracted bark-based dyes scored 3-4 in wet and 4-5 in dry for un-mordanted samples, showing enhanced performance in mordanted samples (4-5 in both), while leaf-based dyes scored 4 in wet and 4-5 in dry for un-mordanted, and 4-5 for mordanted samples

in both conditions; acetone-extracted bark-based dyes scored 4-5 in wet and 4 in dry for un-mordanted samples, with mordanted samples achieving excellent resistance (5 in both), and leaf-based dyes showed moderate resistance in wet (scoring 3) and 4-5 in dry for un-mordanted, with improved scores of 4 for mordanted samples in both; distilled water extraction showed consistent good-to-very good resistance (4-5) in both conditions for bark and leaf-based dyes for both mordanted and un-mordanted samples, indicating that acetone and distilled water are the most effective solvents for achieving superior rubbing fastness, especially for bark-based dyes, while mordanting generally enhances dye adherence and reduces color transfer during use, making dyed fabrics more durable.

Table 5: Showings the Absorbency (A) of the Extracted dye using a wavelength of 550nm

Dye	Granules	Absorbance (A)	Peak	Position (nm)
Ethanol extract	Bark	1.912	1	550nm
	Leave	6.984	2	
Methanol extract	Bark	3.856	3	550nm
	Leave	4.776	4	
Acetone extract	Bark	4.327	5	550nm
	Leave	6.854	6	
Distilled water extract	Bark	4.728	7	550nm
	Leaf	6.736	8	

Ultraviolet/Visible Spectroscopy Measurement

The results indicate the absorbance levels of various dye extracts, with ethanol-extracted leaf dyes showing the highest absorbance value (6.984), followed by distilled water-extracted leaf dyes (6.736), while bark-based dyes extracted using distilled water (4.728) and acetone (4.327)

also show relatively high absorbance. Methanol-extracted bark dyes had an absorbance of 3.856, which is lower compared to other solvents, while ethanol-extracted bark dyes showed the lowest absorbance (1.912) among all bark-based dyes. Across all bark-based dye extracts, the peak absorption consistently occurred at a wavelength of

550nm, indicating that this is the optimal wavelength for these dye types, which suggests a potential similarity in chromophore structure and color properties.

Fourier's Infrared Spectroscopy Measurement

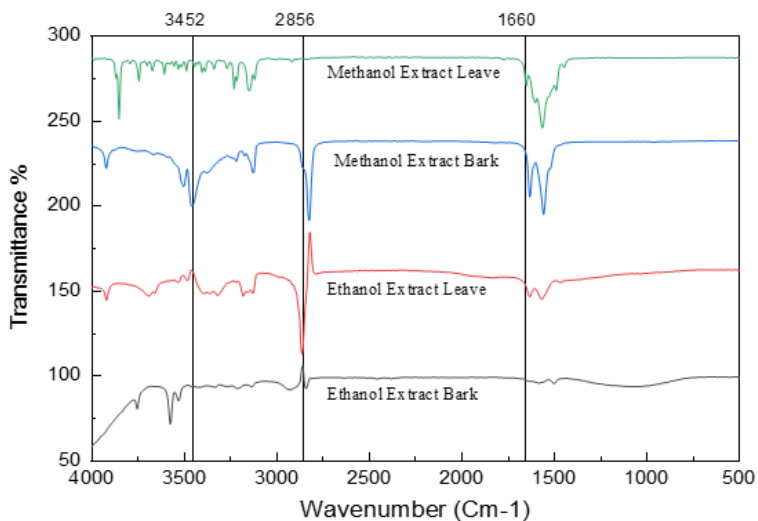


Figure 1.0: FTIR spectra for Methanol and Ethanol extract for both leaves and Bark

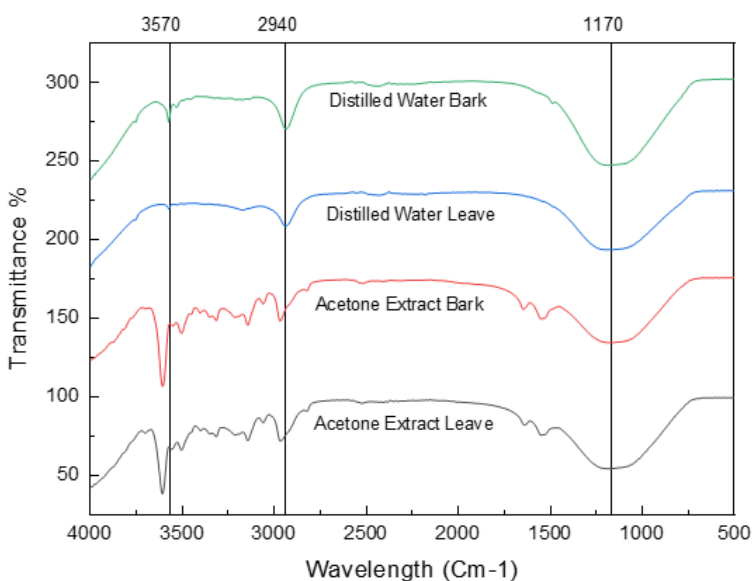


Figure 2: FTIR spectra for Methanol and Ethanol extract for both leaves and Bark

In figure 1.0 the FTIR peak at 3452 cm^{-1} corresponds to O-H stretching, indicating the presence of hydroxyl groups, possibly from alcohols, phenols, or water, commonly found in plant extracts like phenolic compounds, flavonoids, and tannins, with higher intensity in leaves due to their higher water and phenolic content; the peak at 2856 cm^{-1} corresponds to C-H stretching, characteristic of alkanes or lipid components such as fatty acids, with varying intensity depending on the solvent and plant part, as bark typically contains more lignins and waxes; and the peak at 1660 cm^{-1} corresponds to C=O stretching, suggesting carbonyl groups from flavonoids, quinones, or carboxylic acids, which contribute to the dye's properties, with methanol extracts showing stronger

O-H and C=O peaks due to its polarity, while ethanol extracts may highlight less polar compounds, and bark extracts showing more pronounced C=O peaks due to higher tannin and quinone content, while in figure 2.0 The FTIR peak at 3570 cm^{-1} corresponds to O-H stretching, indicating the presence of hydroxyl groups, possibly from alcohols, phenols, or water, commonly found in plant extracts like phenolic compounds and flavonoids, with higher intensity in the leaves due to their higher water and phenolic content; the peak at 2940 cm^{-1} corresponds to C-H stretching, characteristic of aliphatic compounds such as alkanes or lipid components, with varying intensity based on the extraction solvent and plant part, as bark often contains more waxes and lignins;

and the peak at 1170 cm^{-1} corresponds to C-O stretching, indicative of ethers or esters, suggesting the presence of compounds such as polysaccharides, cellulose, or esters, which may be more prominent in the bark due to its lignin content, with acetone extracts potentially showing more non-polar compounds and distilled water extracts highlighting more polar compounds like phenolics.

CONCLUSION

This study demonstrated that Eucalyptus bark and leaf granules are viable sources of natural dyes, with the extraction process and solvent choice significantly influencing the composition and properties of the dyes. FTIR analysis confirmed the presence of essential functional groups such as O-H, C=O, and C-H, indicating that phenolic compounds, flavonoids, and fatty acids contribute to the dye's characteristics. Methanol and ethanol extracts were rich in polar compounds, while acetone extracts highlighted non-polar components. The dyes, which were visibly soluble in water, produced high yields and exhibited a wide range of hues, with eucalyptus bark giving reddish-brown shades and the leaves yielding lemon green to greenish-brown shades. The dyes exhausted well on both pre-metallized and un-pre-metallized cotton fabrics, with deeper shades observed in pre-metallized substrates due to the mordant creating more reactive sites on the cellulose, increasing dye uptake. Fastness tests indicated that the dyes possess very good to excellent wash, light, and rubbing fastness properties. This study suggests that eucalyptus, which is locally abundant, can serve as a high-quality, eco-friendly alternative to synthetic dyes, particularly for application on cellulosic fabrics such as cotton, which is widely cultivated in Africa. Utilizing eucalyptus-based dyes could contribute to job creation, reduce reliance on synthetic dye imports, and conserve foreign exchange, offering economic and environmental benefits.

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