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MORDANT CONCENTRATION EFFECT ON THE COLOUR STRENGTH OF CYANIDIN GLYCOSIDE DYED WOOL

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Abstract: Natural dye is known to have poor fastness qualities and weak shades in colouring fabrics, and to improve fastness qualities and strength of the shades a mordant is needed. This study therefore aims to investigate the effect of different mordants concentration on the colour strength of the ethyl acetate extract of Phyllanthus muellerianus dyed wool. The aqueous filtrate of acetone and water (50/50 v/v) extract was partitioned successively with n-hexane, chloroform, ethyl acetate and n-butanol. The ethyl acetate fraction in ethanol was separated into its various fractions using thin layer and column chromatography that afforded one compound characterized spectroscopically. The sample absorbs at ultra violet and visible region and the FTIR spectra revealed the presence of carbonyl, resonance, and conjugation. GC-MS analysis characterized the base peak and molecular weight of the compounds is 449.11. The 1H NMR spectra of the extract is dominated by the resonances from cyanidin 3- glycosides (cy 3 – gly). Wool (Cashmere wool) swatches were dyed at acidic pH using a cyanidin glycoside dye with different types of mordents at varied concentrations between 2.5, 5, 7.5, 10, 12.5 and 15%. The results of the effect of mordant concentration on the color strength obtained showed that the color strength was optimal for 7.5 -10 % K2Cr2 O7 and 5-7.5 % CuSO4 as mordants concentration. Copper sulphate (CuSO4) mordanted wool fabric has better and more stable colour strength that increases with increase in mordant concentration than potassium dichromate (K2Cr2O7).

Keywords: Natural dye, Phyllanthus muellerianus, cyanidin glycoside, mordant, colour strength.

Introduction

Natural substances capable of colouring natural material fibers such as textiles, food, drugs, cosmetics, candle, leather, paper, plastics, artworks, and building walls are termed natural dye (Kumar Gupta,

2020; Pizzicato et al., 2022; Saxena & Raja, 2014), and applied in solution or dispersion often with the aid of a mordant.

Majority of natural dyes are vegetable-based, and are agro-renewable, bio-degradable, and environmentally friendly with poor fastness qualities and weak shades, unlike synthetic dyes with brilliant colours, affordable, availability, and rich fastness properties but have been reported not to be healthy, carcinogenic, and harmful to the ecosystem, and therefore unsafe. It is ironic that the great invention of synthetic types of dye by a Germany chemist, William Henry Perkin in 1856 was first legislated/banned in Germany (Alam et al., 2020; Ragab et al., 2022; Kumar Samanta, 2020; Prabhu and Bhute, 2012).

As a consequence of the environmental friendliness, and rich health indications, there is a great major switch from the use of synthetic dyes to natural dyes in most applications (Mabuza et al., 2023; Che & Yang, 2022).

Dyeing of natural fabrics and craft works with natural dye is an ancient and domestic household-safe practice. Vocational health challenges eminent with textile and synthetic dye-producing workers include allergic reactions, asthma and other respiratory related problem, cough and chest issues, lung diseases, cancer issues, skin disease and many more due to inhalation, contact, and digestion of harmful chemicals (Islam, 2022; Soyinka, et al., 2024; Okafoagu et al., 2017; Ramadan et al., 2023; Soyinka et al., 2024; Samanta, 2020; Saxena & Raja, 2014; Bishal et al., 2023).

Owing to the poor fastness qualities and weak shades most natural dyes require mordants to fix permanently to the fabric, attain acceptable wash fastness, and improved shades without washing out. Most commonly heavy metal ions and sometimes tannins have characteristic affinity for different fabric producing different colours for each dye, enhancing colour fastness, improving colour vibrancy, and helping natural dye bind to the fibre (Gupta, 2020; Jihad, 2014; Hassan et al., 2024; Benli, 2024; Islam et al., 2024; Pargai et al., 2020).

The regular metal salts used in natural dyeing are alum, iron, copper, chrome, or tin, which, when the effluent water is after the dying process contaminate the environs. The most toxic of the all are chromium and tin. Misused of some of them such as copper and iron can be quite threatening. The safest of them all are alum and is the most popular and non-harmful to man and the environs. Most metal used in mordanting fabrics to improve fastness are not all environmentally friendly (Tchounwou et al., 2012; Repon et al., 2017; Peana et al., 2021; Sundrarajan et al., 2011; Ansari & Iqbal, 2021; Prasad et al., 2021).

Most natural dyes are not substantive (cannot be applied directly to textiles) to the fiber to be dyed. Therefore, a two-step process of first mordanting to make ready the fabric to receive the dye and then the proper application of dye. Mordant should not affect the physical characteristics of the fiber, it only creates a chemical bond where the dye bind (Che & Yang, 2022; Yamini et al., 2022; Pizzicato et al., 2023; Kumar Gupta, 2020; Saxena and Raja, 2014; Vankar, 2017; Tuulik & Beilmann, 2024; Bide, 2021; Samanta et al., 2018).

Material and method

Collection and Processing of Plant Sample

Leaves of *Phyllanthus muellerianus* plant were collected from Agbani near the building of Faculty of Applied Natural Sciences of Enugu State University of Science and Technology. They were dried in a subdued sunlight and ground into powder form with the aid of Kika Grinding Mill (Model MP300-20 A11 basic). The powder form was used to extract the corresponding dyes.

Extraction of Plant Dyes

The powdered leaves material (1.5 kg) was percolated in acetone and water (50/50 v/v) at the ratio of 1:3 for 48 hours and filtered. The acetone was distilled out of the filtrate and the aqueous filtrate remaining was further partitioned successively with n-hexane, chloroform, ethyl acetate and n-butanol. Each partition was concentrated using rotary evaporator (Tena & Asuero, 2022; Pisoschi et al., 2016; Ogbuanu & Amujiogu, 2018; Huynh et al., 2024). The concentrate was dried at 65^{o}C in a Pickstone Thermostatic Oven series 30/300 (Model BD/AL) to a constant weight

Purification of the Plant Dye

The ethyl acetate fraction was of interest and further purified by fractionating with n-hexane, chloroform, ethyl acetate and n-butanol respectively and concentrated.

Modified thin layer chromatographic analysis

A solution of ethyl acetate fraction in ethanol was spotted on the precoated TLC plate (F-245, type E) and the chromatogram developed with benzene, ethyl acetate and acetic acid (12:6:2) (Ajayi, 2010). The chromatogram after development was visualized in iodine tank and by spraying with 10% KOH in ethanol. (Kowalska & Sajewicz, 2022).

Column Chromatographic Separation of Dyes

The column was prepared with chloroform and silica gel. A solution of ethyl acetate fraction (1g in 7.5 mL) methanol was applied on top of the prepared column and eluted with gradient of n-hexane increasing the polarity with ethyl acetate. Eight fractions were collected and TLC analysis conducted. The combined fractions were tested for the presence of flavonoids and anthraquinones (Kowalska & Sajewicz, 2022; Purba & Sembiring, 2022).

UV-Vis spectroscopy analysis

A scan between 190 to 900 nm for characteristic absorption spectra of the fraction was analyzed by placing an aliquot of the dye fraction in a quartz cell and analyzed in a UV/Vis spectrophotometer using Thermo Electron UV spectrometer equipped with vision pro-software's v4.10 (Babatunde, 2017; Sanjayet al., 2018; Espinosa-Morales et al., 2012; Sakshi et al., 2022).

FT-IR spectroscopic analysis

The sample and potassium bromide in the ratio of 1:100 was ground into a fine powder in a Wiggle-Bug. The ground mixture was pressed to pressures of 15,000 psi for about 20 seconds with a hydraulic

press and used to run the spectrum of the sample (Olori et al., 2021; Shepela et al., 2015; Nugrahani et al., 2018; Gopanna et al., 2019).

GC-MS analysis

At linear velocity of 30 cms^{-1} 2 µl of sample was injected at a temperature of 280 ^{o}C. The carrier gas helium at a pressure of 5.0 Pa. s with a flow rate of 40 ml/min was used. The oven was heated from initial temperature of 200 ^{o}C to 330 ^{o}C at a rate of 3 ^{o}C min^{-1} and maintained at this temperature for 5min, the detector operated at a temperature of 320^{o}C. The sample extract (0.5 µl) was injected into the equipment via the injector of the equipment and allowed to scan for volatile components. The peaks obtained were confirmed by comparing their spectra with those of NIST Library mass spectra (Geiger & McElmurry, 2020; Babatunde, 2017; Dilshad Jamadar & Sannapapamma, 2018; Devanand et al., 2024; Degani et al., 2015; Abo-Ayad et al., 2024).

Nuclear magnetic resonance (NMR) analysis

Approximately 20 mg of the dye fraction was dissolved in 0.7 ml of prepared deuterated chloroform $CDCl_3$ for use and filtered to remove any insoluble impurities. The impurities free solution is transferred to a 5 mm NMR tube prior to analysis. All ^{1}H NMR spectra were manually phased, baseline corrected and integrated using VNMR software package VnmrJ2.2D/3.2/4.2A (Agilent Technologies, Inc, USA) (Elyashberg, 2015; Vasil'ev et al., 2024).

Preparation of the wool material for dyeing

All the test fabrics (wool) were cut into 30 x 12 cm and washed for 15-20 minutes with 1 g/L of non-ionic detergent and 0.5 g/L concentrated ammonia at 40-50 ^{o} C. The material was then washed thoroughly with plenty of tap water and soaked in distilled water for 30 minutes and air dried at room temperature (Geelani et al., 2017; Ali et al., 2011).

Dying of Fabrics

Wool (Cashmere wool) samples were dyed using a cyanidin glycoside dye bath containing 3% dye, 8% sodium sulphate and 4% acetic acid at liquor ratio of 1:50. The dyeing of wool was performed at acidic pH by adding the required amount of acetic acid (CH_3COOH). All the test fabrics were dipped in 250 mL of dyeing solution (dye bath) at room temperature. The temperature was gradually raised to boiling point and the dyeing continued at the boiling point for 1 hour. Water was then removed by squeezing the material. (Miljkovic et al., 2014; Moula et al., 2022; Geelani et al., 2017; Ali et al., 2011).

Table 1: Summary of dye bath liquor

1. Dye (EA-1)	3 %
2. Sodium sulphate	8 %
3. Acetic acid	4 %

Note: % is on weight of wool being used.

Extent of the Dye Absorption by the Wool Fabric: Beer Lambert Method

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This was carried out to obtain the amount of dye absorbed by the wool fabric from the dye bath liquor. The dye bath liquor was prepared by dissolving 3% dye in 250 mL of water then 8% sodium sulphate and 4% acetic acid all on weight of wool being used. A dilution series was made to fabricate Standard curve by adding 5 mL of 0, 0.5, 1.0, 1.5, 2, 2.5 and 3% of a dye liquor bath to seven test tubes numbered 0 to 6 respectively. The dilution series were prepared the following way (Table 3.3) (Osharode & Otutu, 2023; Shang, 2013; Musa et al., 2013).

The absorbency of each of the above dilutions (a - f) was determined at 495 nm respectively along with the dye bath liquor after dying and the readings used in the construction of calibration curve (Parekh and Maheria, 2012).

Table 2: Dilution series for fabrication of standard curve

S/N	Dilution	Percent liquor
A	**50 ml of 3 % dye liquor**	3 % dye liquor
B	41.7 mL of 3 % dye liquor + 8.3 mL water	2.5 % dye liquor
C	40 mL of 2.5 % dye liquor + 10 mL water	2 % dye liquor
D	37.5 mL of 2 % dye liquor + 12.5 mL water	1.5 % dye liquor
E	33.3 mL of 1.5 % dye liquor + 16.7 mL water	1 % dye liquor
F	25 m L of 1 % dye liquor + 25 m L water	0.5 % dye liquor

Extent of the Dye Absorption by the Wool Fabric: Spectrometric Method

Dye concentration in the dye bath was measured at the start, and after dyeing to determine the exhaustion which is the percentage of dye absorbed by the fabric (Safi & Amirshahi, 2023; Rahman Bhuiyan et al., 2016; Khatri & White, 2015; Hamdaoui et al., 2013; Cay et al., 2009). The absorbency was measured spectrophotometrically using 75 series Single Beam UV-Visible at a wavelength of 590 nm λ_{max} to determine the concentration of the dye. The percentage of dye bath exhaustion was calculated according to equation (1) below:

$$\%\,E = \left(\frac{AO - Ad}{AO}\right) x\ 100\% \qquad (1)$$

Where A_o is the absorbance of the dye initially in the dye bath and A_d the absorbency of residual dye in the dye bath after some time. (Nandiyanto et al., 2023; Efeze et al., 2024; Safi & Amirshahi, 2023; Hossain et al., 2020).

Mordanting of Fabrics

The post mordanting were employed with different mordants (copper sulfate, and potassium dichromate) at varied concentration (2.5, 5, 7.5, 10, 12.5 and 15%), at optimized mordanting conditions using 1% acetic acid. The process of wool mordanting was started at 60 ^{o}C and slowly increased to boil with gentle stirring and continued for 1 hour. Then was left to cool down to room temperature and rinsed in running tap water until the colour became clear. The fabric was thereafter squeezed and kept for drying (Poornima & Sujatha, 2023; Hossain et al., 2015; Haar et al., 2013; Wanyama et al., 2010; Lodrick et al., 2015; Geelani et al., 2017; Ali and El-Mohamedy, 2011).

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Table 3: Summary of mordant solution composition for mordanting of fabrics.

Mordanting Chemical	% Composition					
$K_2Cr_2O_7$	2.5	5	7.5	10	12.5	15
$CuSO_4$	2.5	5	7.5	10	12.5	15
Acetic acid	1	1	1	1	1	1

Colour strength

The reflectance of the samples was measured on a spectrophotometer using 75 series Single Beam UV-Visible. Relative colour strengths (K/S values) were determined adopting the Kubelka–Munk equation *(2)* (Safi & Amirshahi, 2023; Hossain et al., 2018; Myrick et al., 2011; Li et al., 2022; Khatri et al., 2014).

$$K/S = \frac{(I - R)^2}{2R} - \frac{(I - R)^2}{2R_0} \qquad (2)$$

Where R = decimal fraction of the reflectance of dyed fabric,
R_o = decimal fraction of the reflectance of undyed fabric,
K = absorption coefficient, and S = scattering coefficient.

Results and discussions

Percentage Yield of Crude and Purified *Phyllanthus muellerianus* plant Leaves Dyes

Out of 1.5 kg of plant material, 1.76% was obtained as the crude plant dye. Ethyl acetate fraction of plant dye of the leaves of ***P. muellerianus*** plant gave high content (1.15%) than n-butanol fraction (0.61%). The three fractions obtained from the ethyl acetate fraction were EA-1 (0.81%), EA-2 (0.64%) and EA-3 (0.17%). (Table 4.2).

Table 4: Percent yield of crude and purified dyes in the leaves of P. muellerianus plant

Solvent partition	Plant dye (g) & (%)	Purified dye (g) & (%)		
Ethyl acetate	17.24 (1.15%)	Fractions		
		EA-1	EA-2	EA-3
		0.14 (0.81%)	0.11 (0.64%)	0.03 (0.17%)
n-Butanol	9.22 (0.61%)	-		

The thin layer and column chromatographic analyses was to know the expected number of components and obtain pure products for spectroscopic analysis from plant dye EA1. The three spots obtained from thin layer chromatography gave R_F values of 0.16, 0.38 and 0.60 respectively.

Further column chromatography analysis gave eight fractions that were separated by thin layer chromatography using 12:6:2 benzene/ethyl acetate/acetic acid as eluent. Examination of the chromatogram under iodine vapour and 10% potassium hydroxide showed that fractions A and B, C and D and G and H (Plate 1) were the same and combined so. Fraction F is distinct. The combined fractions and fraction F were tested for flavonoids by treating sample with few drops of Ferric chloride solution and the formation of blackish red colour indicating the presence of flavonoids. Also, by treating with sodium hydroxide solution, the increase in the intensity of yellow color that become colorless on addition of few drops of dilute Hydrochloric acid, indicates the presence of flavonoids.
The results obtained from the spot analysis for flavonoids on fractions F and GH were positive and the fractions evaporated to a constant weight using rotary evaporator (Model RE-52 CS).

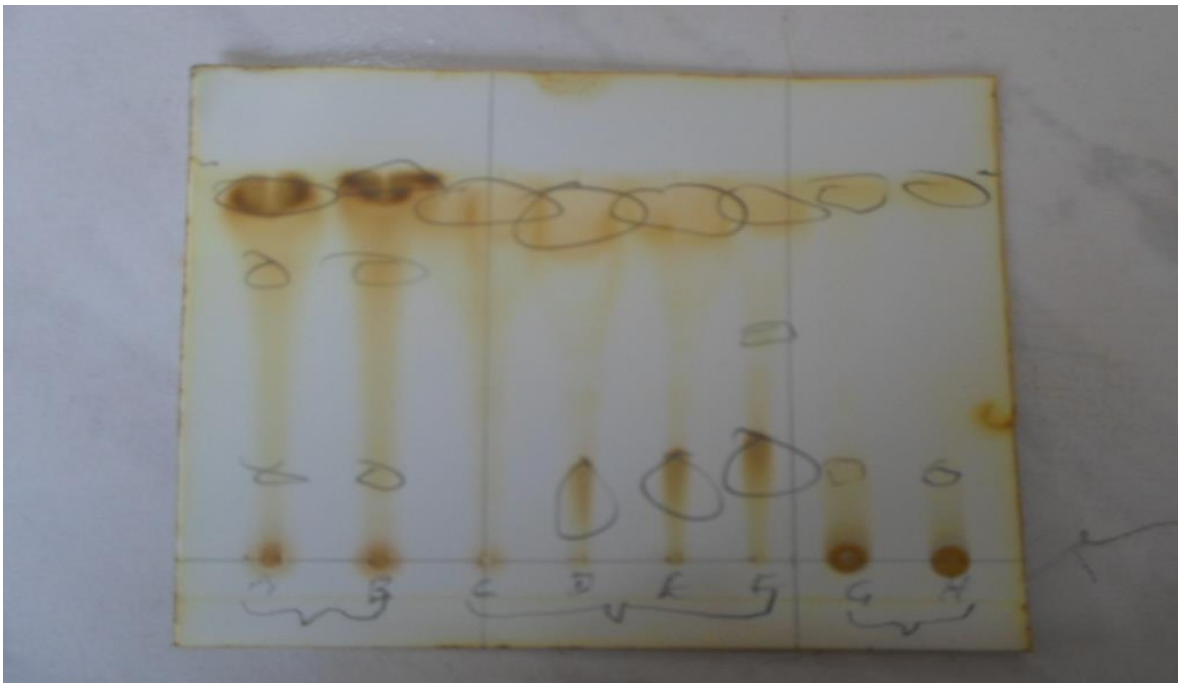

Plate 1: Result of TLC analysis of the eight fractions

The UV/visible spectra of the F1 sample revealed that it absorbs at (313 and 482 nm) and 747 nm) (Fig. 2). The F1 dye of ***P. muellerianus*** plant leaves going by the UV/ visible absorption at the ultraviolet region (313 nm) will probably confer excellent protection against UV-A to B radiation thereby preventing penetration UV-A radiation to the skin and development of cancer.
Most plant natural dye shows absorbance in the ultraviolet region but with exception of a few in the visible region that usually occurs in between 465 and 800 nm (Scarano et al., 2020; Bhim et al., 2014; Paudel et al., 2018; Karuppaiah et al., 2024; Ezeokonkwo et al., 2018; Oliveira et al., 2024). The F1 dye also shows maximum absorption in the visible region at 482, and in 747 nm and as such can be considered a dye.

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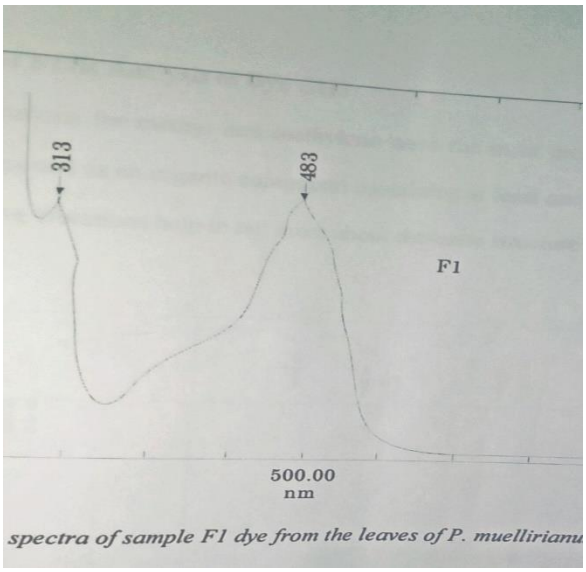
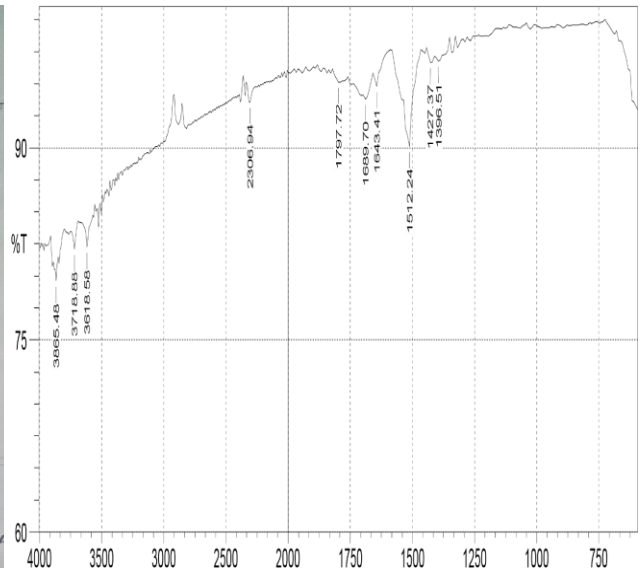
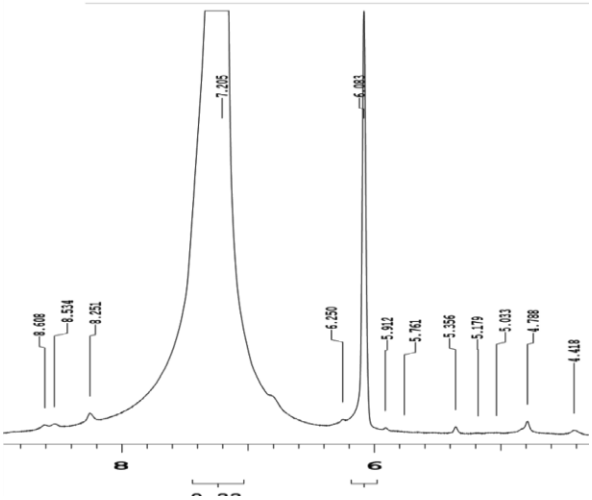
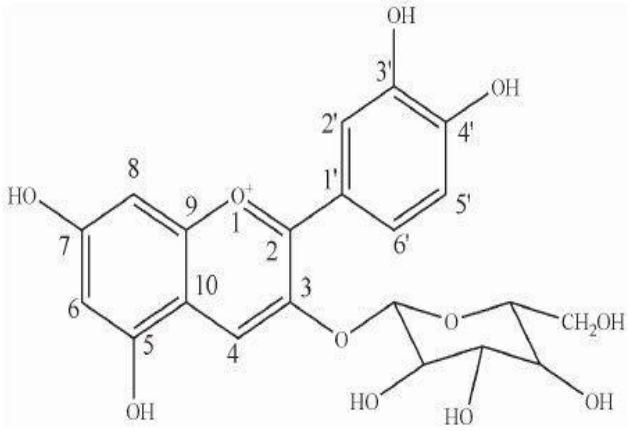


spectra of sample F1 dye from the leaves of P. muelliriana



The FTIR result shows the presence of at least one chromophore (ethylene, carbonyl, acids, esters, etc.) that qualifies the extract to exhibit dye property (Gürses et al., 2016; IARC, 2010; Britannica, 2024; Kumar et al., 2021; El Sikaily et al., 2012).





The spectra of the F1 dye are shown in Fig. 4.3. The spectra of the sample are suggestive of the presence of an anthocyanin, with aromatic signal in the 6-9 ppm region (Tulio et al., 2008 and 2007).
The signal in sample F1 (8.6, 8.5 and 8.3 ppm) could be due to the presence of aromatic protons of an anthocyanidin. In the spectrum of cyanidin - 3, 5 – glycoside used as a standard, there is a singlet at 9.1, a doublet at 8.3, a doublet at 8.0 and a doublet of doublets at 7.0 showing protons on the 4, 6′, 2′ and 8 carbons respectively, of the cyanidin - 3, 5 – glycoside. The assignment of these peaks corresponds well to the literature values – having H-4 at 8.81 and 9.03 as a singlet, H-6 as a doublet of

doublets between 8.06 and 8.35, H-2 as a doublet between 7.83 and 8.13, and H-8 as broad singlet at around 6.98 (Shoji et al., 2002; Byamukama et al., 2005).

For the fact that none of the peaks in the spectra of the sample marched any of the one from the standard, it can be concluded that the standard and the sample is a different compound. The 1H NMR spectra of the extract is dominated by the resonances from cyanidin 3- glycosides (cy 3 – gly).

P. muellerianus plant leaves isolate can be considered a dye because it absorbs in the visible region (400–700 nm), contain at least one colour bearing group (chromophore), carries alternating single and double bond (conjugated system) which increases intensity with increase in the number, and exhibit mesomerism bonding system which is a measure of stability (Inanabor Isibor, 2024; Ogbuanu et al., 2018).

The calibration of a standard curve for P. muellerianus plant Leaves dye liquor (%/L)

The stock solution of the dye was prepared in water because of its comparatively low volatility and availability and from this, a series of dilution was made. The absorption spectrum of each of the dilution was obtained. A graphical plot of absorption versus concentration of these solutions yielded a straight line, (Fig. 1), indicating adherence to Beer's law. The extinction coefficient obtained was 5.6818.

Table 5: The absorption spectrum for standard curve for P. muellerianus plant leaves dye liquor (%/L)

Dye liquor (%/L	Absorbency
0.1	0.334
0.2	0.336
0.3	0.341
0.4	0.352
0.5	0.355
0.6	0.357

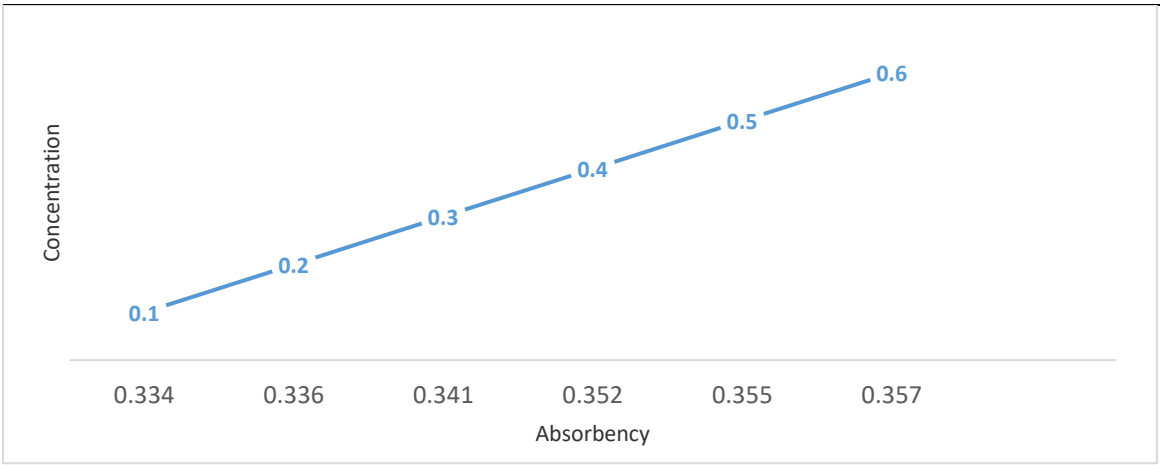


Fig. 1: Standard curve for P. muellerianus plant leaves dye liquor (%/L)

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Effect of mordant concentration on the colour strength of P. muellerianus plant leaves dye dyed wool

The optical density of wool fabric with various concentrations of $CuSO_4$ and $K_2Cr_2O_7$ was measured against the undyed wool samples in the Perkin-Elmer Lambada 3B UV/Vis spectrophotometer. The values of color strength (K/S) values were determined by the Kubelka Munk equation (Geelani et al., 2017; Jabar et al., 2021; Morshed et al., 2022; Manyim et al., 2021). One of the most significant differences between colour strength of dyed wool with $CuSO_4$ and $K_2Cr_2O_7$ was that $CuSO_4$ has more stable colour strength than $K_2Cr_2O_7$. This probably is due to the ability of the dye molecules to aggregate inside wool fiber, the dye and also the type and concentration of mordant used (Indira et al., 2024; Bulut et al., 2014).

Table 6: Effect of mordant concentration on the colour strength of *P. muellerianus* plant leaves dye dyed wool

Mordant concentration (%)	Colour strength	
	$K_2Cr_2O_7$	$CuSO_4$
2.5	0.87	1.35
5	0.88	1.38
7.5	0.90	1.41
10	0.95	1.47
12.5	1.03	1.60
15	1.15	1.81
Without mordant (0)	0.69	

The comparative absorbency of various dilutions of the dye using Perkin-Elmer Lambada 3B UV/Vis spectrophotometer was measured spectrophotometrically at λmax 550 nm. Table 4.6 and Figure 3 illustrate that as the dye concentration increases, more radiation is absorbed thereby increasing the absorbance. The result of the study indicated that, the concentration and absorbance are directly proportional (Barzan. & Hajiesmaeilbaigi, 2018; Minó et al.,2023; Aldabib & Edbeib, 2020).

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In the post mordanting process of wool employed in this study, copper sulphate and potassium dichromate gave degrees of dark brown khaki (carton) colour on wool at different concentrations.

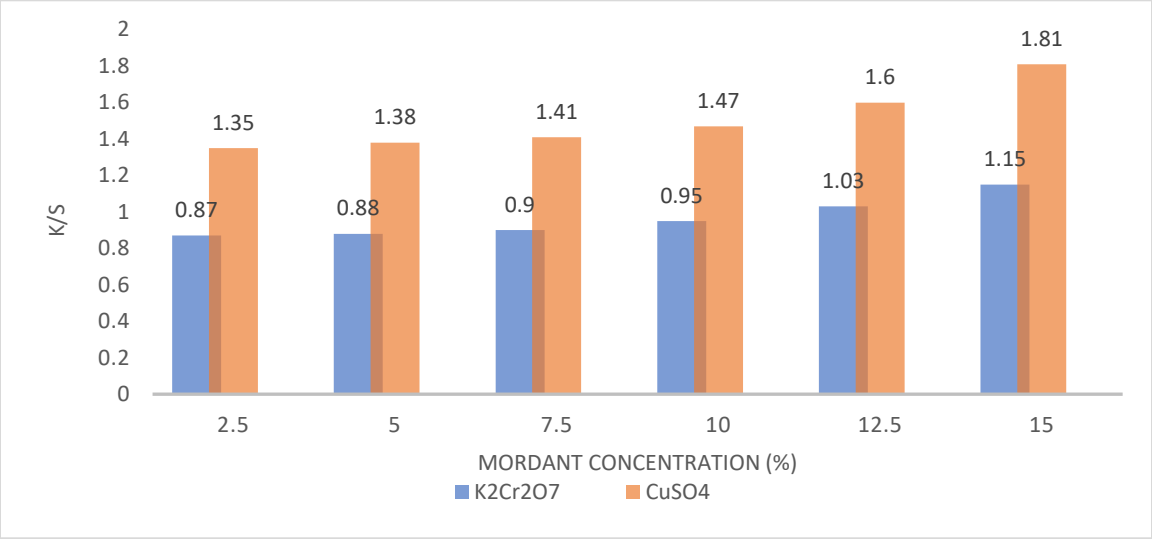

Fig. 3: **Effect of mordant concentration on the colour strength of *P. muellerianus* plant leaves dye-dyed** wool.

While alum and tin chloride gave a unsignificant faint brown colour on wool dyed with cyanidin glycoside. Alum ($K(Al)(SO_4)_2$) and tin chloride are generally considered a neutral mordants that give no appreciable colour different than that of dye bath and brighten colours a bit respectively. This, however shows that the dye did not bond well with the use of alum and tin chloride as mordants like that of copper sulphate and potassium dichromate (Wanyama et al., 2010; Prabhu & Bhute, 2012; Tera et al., 2012;). This agrees with Sahoo et al (2017), that mordants are more important than the dye itself in determining the dye fixation and colour to the fabric.

Copper sulfate produced brighter, more vibrant colors than other potassium dichromate. Both mordants changed the color of natural dyes, light yellow into brown (carton colour), with copper

sulphate giving deeper brown shade (hue) that has better washability than potassium dichromate (Wanyama et al., 2010; Prabhu and Bhute, 2012; Otutu et al., 2019; Indira et al., 2024; Miah et al., 2017).

Conflict of interests

The author(s) did not declare any conflict of interest.

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