Exploring the Potential of *Dacryodes Edulis* Leaf Extract as Natural Colourant on Polyamide Fabrics: Extraction, Characterization and Application

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Abstract:

There is a growing demand for sustainable and eco-friendly alternatives in the textile industry, particularly in search of natural colourants derived from plants. This research study investigates the extraction and characterization of natural colourant from the leaf of *D. edulis* and explores its application to polyamide fabrics. Laboratory experiments, such as solvent extraction was performed to obtain the colourant. The extraction process was optimized using Response Surface Methodology-Central Composite Design (RSM). The dye was isolated and characterized using vacuum liquid chromatography, UV-Vis spectrophotometry, high-performance liquid chromatography and Fourier-transform infrared spectroscopy. Physical properties such as light fastness, perspiration fastness, rubbing fastness and wash fastness were assessed to determine the durability and stability of the natural colourant on the fabrics. The results indicated that the optimal conditions for dye extraction are 65.9 ℃ and 2 hours, providing feasible parameters for replication. Examination of the natural dye obtained from the isolated fraction, revealed the presence of carbon-carbon double bonds (C=C), carbonyl (C=O), ester (-COO), aldehyde (CHO) and hydroxyl (-OH) groups; with rutin, isoquercetin and tannic acid as its major compounds. The isolated dye exhibited an absorption peak at 403 nm. The ratings for the treated fabrics varied from fair to good and very good. The light fastness ranged from 4 to 6, perspiration fastness and wash fastness ranged from 2-4, and rubbing fastness ranged from 3-5. On the other hand, the untreated fabrics had ratings, with a range of 2-5 for light fastness, 2-4 for perspiration fastness and wash fastness and 1-4 for rubbing fastness, it was also observed that the colour strength values of mordanted fabrics were deeper than those of the unmordanted fabrics. These results indicate that the dye obtained from *D. edulis* leaf has considerable potential as a source of natural dyes. These findings contribute valuable insights into the extraction, characterization and application of natural colourants, promoting a shift towards more sustainable and eco-friendly practices in the textile industry.

Keywords: Natural dye, Extraction, Characterization, Response surface methodology, *Dacryodes edulis*, leaf, Polyamide, fabrics

1. Introduction

Humanity has progressed from the stone age to the information age and is now making deep inroads into the environmental age, thus, there is a growing interest in natural dyes [1]. These dyes, derived from plants, animals

or minerals without chemical processing, come from various parts of plants and insects [2]. While the invention of synthetic dyes in 1856 led to a decline in natural dyes [2], synthetic dyes have notable drawbacks, including carcinogenic properties and allergic reactions. In contrast, natural dyes have numerous advantages such as being eco-friendly, non-toxic, and aesthetically pleasing and even possessing medicinal properties [3]. This has further fueled interest in natural dyes for textile colouration, especially in countries with strict environmental regulations [4].

The dyeing process of natural dyes can be significantly improved using a Soxhlet apparatus which allows for the efficient extraction of concentrated dye solutions from natural sources. This method is advantageous as it recycles the solvent, producing a more potent dye extract and minimizing waste [5]. Studies have shown that the Soxhlet extraction method can yield higher dye concentrations, which in turn can lead to better colour yield and fastness properties on the dyed textiles [5].
In industrial processes using natural dyes, it is crucial to characterize these compounds efficiently and non-destructively. Fourier-transform infrared spectroscopy (FTIR), High-performance liquid chromatography and UV-vis Visible spectrophotometry are commonly employed techniques due to their affordability, ease of use, reliability and ability to provide detailed data [5; 6].

Natural dyes exhibit distinct properties and affinities for different fibers which can be enhanced through various dyeing techniques. For instance, indigo, derived from the Indigofera plant, is renowned for its vibrant blue hue and finds common application in wool and nylon textiles [7]. Nylon, being a synthetic polyamide with a structure resembling protein fiber, can benefit from modified dye baths containing additives that enhance dye affinity for synthetic materials. Onion extract, derived from the outer papery skin of onions, contains flavonoids and tannins, serving as a natural dye. Onion dyeing yields vibrant shades and possesses antibacterial properties [8]. Lac insect is another natural dye used for wool and nylon. Its rich, deep hues enhance colour and fastness properties in these fabrics [9]. Madder, a plant-based dye, imparts a vivid red colour and similarly improves colour and fastness in wool and nylon fabrics [10]. These natural dyes offer sustainable alternatives to synthetic dyes, thereby reducing environmental impact and promoting eco-friendly textile production.

One promising natural dye source is *Dacryodes edulis* (G. Don) H. J Lam (Figure 1), also known as safou in the Republic of the Congo and Angola, plum in Cameroon and Ube in Nigeria, is a native fruit tree primarily found in the humid lowlands and plateau regions of West, Central Africa and Gulf of Guinea countries. This evergreen tree can grow up to 18- 40 meters in the forest but typically stays under 12 meters in plantations Along with producing edible fruits, the bark, leaves, stems and roots of *Dacryodes edulis* are used as local medicine for treatment of certain diseases [11]. Since natural dyes have been the subject of extensive research, including the extraction and application of natural dyes from orange peel and lemon peel on cotton fabrics, as well as the extraction of natural dyes from selected plant sources and its application in fabrics [3, 12]. This study explores the extraction, characterization and potential application of *D. edulis* leaves as a natural dye source.

Figure 1: ***Dacryodes edulis*** **leaves**

2. Materials and Methods

2.1 Chemicals and reagents

Laboratory grade ferrous sulphate ($FeSO_4$) was used as mordant while a diluted solution (2 g/L) of sodium carbonate (Na_2CO_3) was used to adjust the pH of the dye solution to 7. Reference detergent A (ECE phosphate-free standard detergent powder wfk-Testgewebe GmbH) soap (5 g/L) was used for the wash fastness test. Hydrochloric acid (37% fuming HCl), *n*-hexane, acetonitrile, ethyl acetate, glacial acetic acid, ethanol, Wagner's reagent (potassium iodide and iodine crystal) and sulfuric acid, all of which were of analytical grade and obtained from Merck (Darmstadt, Germany) were utilized in this study.

2.2 Plant material

The plant material used in this research was *Dacryodes edulis* leaves (Figure 1) collected as discarded waste from households in Kiagbodo, Delta State. The leaves were identified and given a voucher number (No. 0614) by Dr. B. E. Omomoh of the Department of Forestry and Wood Technology at the Federal University of Technology, Akure, Nigeria. The leaves were washed with distilled water without squeezing to remove debris and dust particles and then dried in the sun for a period of three days, as exposure to UV radiation can enhance colour development through photooxidation processes [13]. Once thoroughly dried, the leaves were pulverized into a powder, using a manual blender (Porkert Manual Grinder No.32), and stored at room temperature until further use.

2.3 Optimization of extraction parameters

Preliminary Soxhlet extraction was carried out while varying two parameters (solvent and temperature). Four solvents namely: ethanol, dichloromethane, 0.5 M sulphuric acid with ethanol and distilled water, were used. Briefly, the colourant was extracted using 200 ml of each solvent, which was placed in a round bottom flask, heat was supplied through a heating mantle to the flask and 10 g of the powdered waste leaves was held in a paper thimble which sat in the Soxhlet apparatus. Extraction was done at different temperatures (40, 50, 60, 70, 80 and 90 °C) for one hour each. Thereafter, the extracts were allowed to cool down and an aliquot of each was analyzed using a UV-vis spectrophotometer to obtain the absorbance.

Statistical analysis was conducted using a Central Composite Design [14]. Optimization was performed using five levels: -α, -1, 0, +1 and +α (Table 1). A total of 13 experiments were conducted, with two independent variables: temperature (X_1) and extraction time (X_2) to check the reproducibility. To describe the behaviour of the crude dye system, an empirical second-order polynomial mode was used, and its coefficient was determined using Equation 1.

$$Y\% = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1^2 + \beta_4 X_2^2 + \beta_5 X_1 X_2 \quad (1)$$

Where: Y is the response variable or output; β_o, β_1, β_2, β_3, β_4, β_5, are the coefficients of the model that need to be estimated while X_1 and X_2 represent the interaction terms of the independent variables. All analytical tests were

carried out in triplicate. Statistical analysis was performed using the MODDE software. Data were analyzed using the analysis of variance (ANOVA), and a *p*-value lower than 0.05 was considered significant in surface response analysis.

Table 1. Experimental range and levels of independent process variables

Parameter	Symbol	Coded levels				
		-α	**-1**	**0**	**+1**	**+α**
Temperature (℃)	X_1	65.9	70	80	90	94.1
Extraction time (Hr)	X_2	0.6	1	2	3	3.4

The optimal extraction parameters obtained from the response surface methodology-central composite design were used to carry out a second batch of extraction using a Soxhlet apparatus [15]. Thereafter, the extract so obtained was then separated using vacuum liquid chromatography.

2.4 Isolation procedure

Vacuum liquid chromatography (VLC) was performed using the method described by Paranagama and Ndukwe *et al.* with slight modifications (Figure 2) [16,17]. To ensure optimal packing density, the VLC column was dry packed with thin layer chromatography (TLC) grade silica under vacuum. Subsequently, ethanolic crude extract of *Dacryodes edulis* leaves was prepared, along with silica gel mesh, and loaded onto the column. The elution process was carried out by sequentially using 300 ml of suitable solvent mixtures (mobile phase), beginning with a low polarity solvent (100 % *n*-hexane); subsequently, the polarity was gradually increased by adjusting the solvent ratio (*n*-hexane-DCM in ratios of 3:1, 2:2, 1:3), 100% DCM, DCM-ethyl acetate (3:1, 2:2, 1:3), 100 % ethyl acetate, ethyl acetate-EtOH (3:1, 2:2, 1:3) and 100 % EtOH) between each fraction collected. The column was pulled dry after each mobile phase to ensure proper separation.

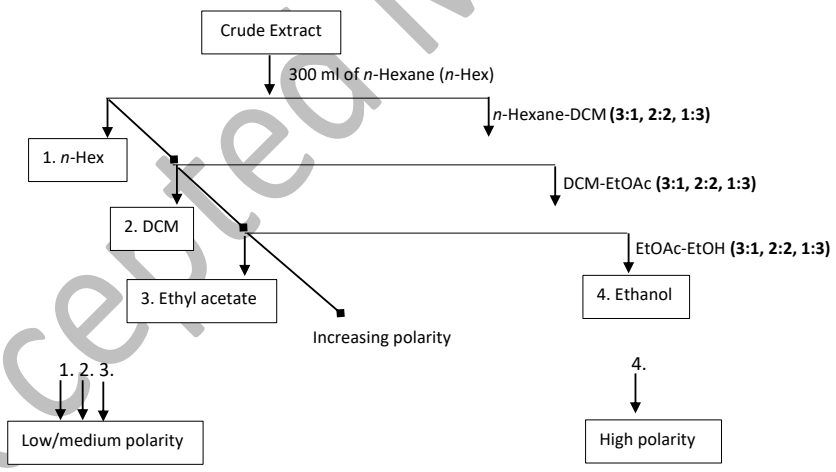


Figure 2. Framework of VLC model

2.5 Phytochemical screening tests

The phytochemical examination was conducted on the isolated dye of *D. edulis* leaves using standard procedures to detect the following bioactive compounds: alkaloids, flavonoids, glycosides, terpenoids, tannins and steroids [17,18].

2.6 UV-visible analysis

The UV-VIS-NIR scanning spectrophotometer UV-3101PC (Shimadzu) was used for all spectrophotometric measurements. All measurements were carried out using quartz cells 10-mm at room temperature (25±2 ℃) and changes in their absorption (400-800 nm) were noted.

2.7 HPLC analysis

The HPLC analysis was carried out using Agilent 1260 infinity HPLC system with a photo diode array detector (Agilent Technologies, Palo Alto, CA). The chromatographic separations were carried out using an Xbridge™ Shield RP_{18} column (4.6 mm I.D. × 150 mm, 3.5µm) (Waters, Milford USA), with column oven temperature maintained at 20 ℃ [19]. The mobile phase comprised of 0.1 % acetic acid (Solvent A) and 100 % acetonitrile (Solvent B). The mobile phase flow rate was 1.0 ml/min with gradient elution. The percentage composition of Solvent B was maintained at 20 % for 3 min, gradually increased to 38 % for 24 min, further increased to 90 % for 1 min and maintained at 90 % for 5 min, followed by equilibration to the initial composition for 6 min. the injection volume was 10 µL and UV absorbance was monitored at 365 nm.

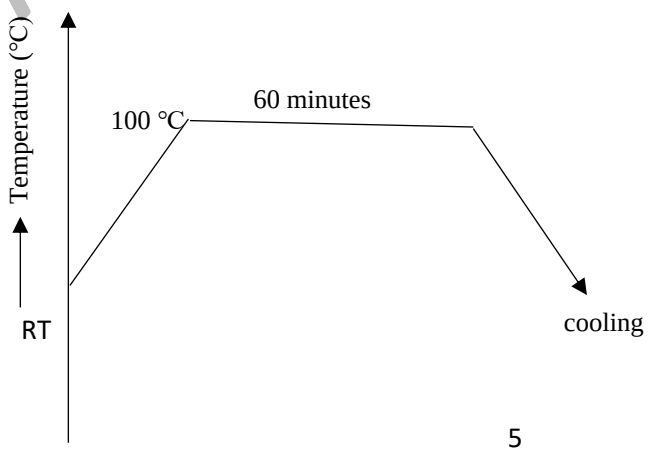
The software used for FTIR data collection was the Infrared Data Management (IRDM) system. The infrared spectrum was recorded at room temperature with a PerkinElmer Fourier Transform Infrared Spectrometer, Model spectra 100 series (Perkin-Elmer Corporation, Norwalk, CT, USA), equipped with a deuterated triglycine sulfate (DTGS) detector and controlled by a Perkin-Elmer PC. The instruments were maintained in constant humidity to minimize water vapor interference. Drops from each standard were placed on the attenuated total reflection element and scanned. After each scan, the ATR diamond was rinsed three times with acetone and dried with soft tissue before adding the sample; Calibration spectrum was obtained from 64 scans at a resolution of 2 cm^{-1} with strong apodization through 3500-1000 cm^{-1} frequency region. The spectrum was rationed against the background air spectrum. All the scans were done in triplicate with the spectrum recorded as absorbance and stored on a disk.

2.8 Preparation of the fabrics

The use of fabrics such as wool and nylon 6 were selected for this study due to their compatibility with natural dyes [20] with wool being particularly sustainable. Nylon 6 and wool were immersed in a detergent solution for approximately 60 minutes and then thoroughly rinsed with tap water until all detergent was removed. Afterwards, the clean fabrics were washed with de-ionized water, gently squeezed and dried in an air oven at 60 ℃. Finally, they were stored in a vacuum desiccator, ready for use.

2.9 Mordanting

In this experiment, the mordanting process was conducted before dying, referred to as pre-mordanting. The aim of pre-mordanting was to enhance the adsorption of the dye and ensure a strong bond between the dye and the fabric. The commonly used mordant, such as ferrous sulphate ($FeSO_4$) was selected. Initially, the wool and nylon fabrics was immersed in warm water (approximately 46 ℃) for 30 minutes to relax the fibers, making the fabrics more receptive to mordanting and dyeing. 8 fabric samples were taken for mordanting. 10 gm/ l $FeSO_4$ was taken in dye pot separately and it was heated with the samples for 1 hour at a temperature of 100 ℃. After 1 hour the fabrics were let for conditioning for 24 hours. After washing these samples are dried (Figure 3).



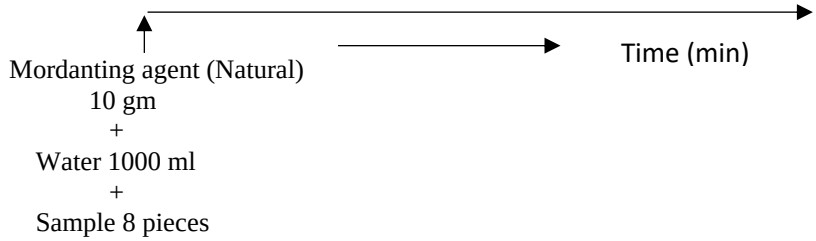


Figure 3. **Process curve for mordanting with natural mordant**

2.10 Dyeing procedures

The dyeing procedures were performed following the general dyeing method [21]. A fabric-to-dye ratio of 1:10 was chosen based on the weight of the fresh natural dyes extracted and the fabrics used in the experiment. The fabric was immersed in a dye bath composed of 0.25% aqueous solution of the dye. The dye liquor ratio of 1:10 was kept constant for all samples, and the pH value of the dye bath was optimized depending on the type of raw material. For *D. edulis*, the pH values were adjusted by adding drops of sodium hydroxide or hydrochloric acid to achieve pH levels of 9-10 and 3-4, respectively. The temperature of the dye bath was gradually increased (about 1 ℃) until it reached 100 ℃ and was kept at this temperature for about 60 minutes. Afterwards, the dye bath was allowed to cool to around 60 ℃. The dyed fabric was then squeezed, thoroughly rinsed with water and air-dried.

2.11 Determination of wash fastness of dyed samples

The dyed specimens of wool and nylon fabrics with a dimension of 5 cm × 4 cm were placed between two pieces of undyed white fabrics of the same dimension. Three pieces were stitched together around the edges to create a composite specimen. The composite specimen was agitated with ten steel balls in a 100 ml beaker, containing a solution of 5 g/L soap and 2 g/L soda ash with a liquor ratio of 1:50 as stipulated by ISO 3 standard (ISO 105-C10:2006). The washing process was carried out at 60 ± 2 ℃ for a duration of 30 minutes in a launder-o-meter. The composite specimen was then rinsed, separated and dried. The change in colour of the test samples and the staining of the adjacent undyed white fabrics were evaluated using the grey scale, with references to the ISO 9001 2000 group.

2.12 Determination of light fastness

Strips of the fabrics and the blue wool standards were cut and mounted on cardboard paper. Half portions of the specimens were covered to obstruct the light source from getting to that portion. The specimens were exposed to natural daylight in a south-facing direction at an angle of 45 ℃, sloping from the horizontal, for a duration of 72 hours in accordance with ISO105B01:2014 standard. After 72 hours, the specimens were removed and the extent of their fading was assessed by comparing them to the blue wool standards.

2.13 Fastness to perspiration test

The fastness to perspiration test evaluates the ability of textile fabrics to resist colour fading or running when exposed to perspiration. This test was carried out according to ISO 105-EO4 standard method, two perspiration solution (acidic and alkaline); the acidic solution consists of sodium chloride (NaCl, 5 g/L), disodium hydrogen orthophosphate dehydrates (Na_2HPO_4 2.5 g/L) and histidine monohydrochloride monohydrate. The pH of the solution was adjusted to 5.5 while the alkaline solution consists of $C_6H_9O_2N_3.HCl.H_2O$ (0.5 g/L) and is adjusted to pH 8 using 0.1 N sodium hydroxide (NaOH). The liquor ratio for the test was 20:1.

2.14 Determination of fastness to dry and wet rubbing

The dyed samples' dry and wet rubbing fastness was tested using a Crock meter in accordance with ISO 105-X 12:2001 standards. The specimen was placed in the Crock meter and a piece of standard white cloth (starch free 96.100 cotton fabric of a long type) was used to rub against the coloured specimen. This rubbing process was carried out under controlled condition of pressure and speed. For both the dry and wet tests, the rubbing fingers were covered with white cloth and moved back and forth for a total of 20 rubbing strokes. The colour transferred onto the white cloth was compared with a Grayscale for alteration of colour, consisting of grades 1-5.

2.15 Evaluation of color strength

Estimation of colour strength of the dyed fabrics are carried out by determining the K/S values using a computer colour matching system (CS-5, Applied colour system, USA). The value of reflectance (R) in the visible wavelength region is measured using the ACS spectrophotometer. The value of reflectance (R) of the dyed fabric is measured at the wavelength of 420 nm and also the K/S value of the sample is found directly from the instrument. Every dyed sample is measured in the same way and the K/S values are obtained directly from the instrument, which follows the Kubelka-Munk theory as in equation (1).

$$\frac{K}{S} = \frac{(1-R)^2}{2R} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

3. Results and Discussion

The research was carried out to ascertain the chemical constituents of the dye from *D. edulis* leaf, which would help identify the compounds responsible for the colouration. Analytical techniques (HPLC, FTIR and UV-vis spectroscopy analysis) were employed for this purpose. The isolated dye was applied to polyamide fabrics, along with additives like mordant, and the dyed fabric was tested for wash fastness, light fastness and perspiration fastness.

3.1 Optimal extraction condition

The process of optimizing experimental conditions, such as the temperature of the extraction process, is crucial for the efficient extraction of target compounds. In this study, the Soxhlet apparatus was optimized to achieve the desired results. The temperature of the extraction process was carefully adjusted within a range of 30 ℃ to 90 ℃. The impact of this temperature variation on the yield of colouring compounds is depicted in Figure 4. The graph presented a clear narrative of the experimental results. The results revealed that ethanol consistently produced higher yields of colourants from the leaf of *D.edulis* when compared to the other solvents. As a result, it was concluded that ethanol is the optimal choice for extracting natural colourants from *Dacryodes edulis* leaf. Additionally, the efficiency of water and dichloromethane were found to be lower than the mixture of ethanol and 0.1 M H_2SO_4 (1:1). The difference in efficiency could be attributed to the acidic nature of H_2SO_4, which helps break down the dye molecules, enhances the solubility of certain compounds, and facilitates the extraction process. The findings of this study, support the research conducted by Al-Alwani et al. [22], who compared the effectiveness of nine solvents for extracting natural dyes from cordyline, pandan and dragon fruit (*Cordylline fruiticosa, Pandannus amaryllifolius* and *Hylocereus polyrhizus*). The solvents tested were n-hexane, ethanol, acetonitrile, chloroform, ethyl ether, ethyl acetate, petroleum ether, n-butyl alcohol and methanol. The study aimed to assess the optimal extraction conditions. The results indicated that ethanol and methanol were the most suitable solvents.

Figure 4 demonstrates that the extraction efficiency of the ethanolic extract remained relatively constant between 30 ℃ and 40 ℃. However, a marked increase in colour yield was observed from 40 ℃ to 70 ℃, suggesting that temperature plays a significant role in the extraction efficiency of colouring compounds, a finding that aligns with previous research [23]. However, a slight yield reduction beyond 70 ℃ suggested possible pigment degradation in certain dyes at excessively high temperatures [21].

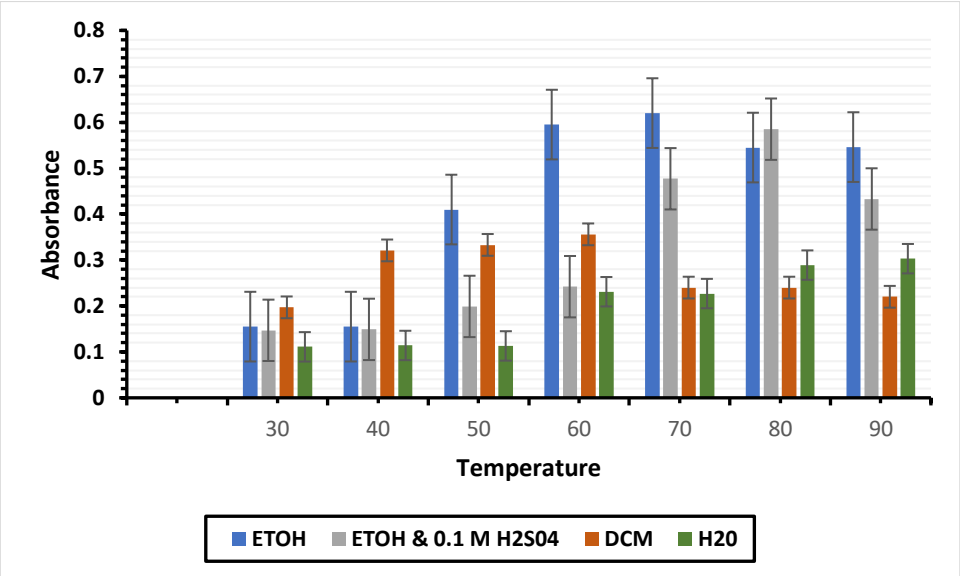


Figure 4. Effect of Temperature on the Concentration of *D. edulis* leaves Dye when Extraction Solvents (Mixture of Ethanol and 0.1 M H_2SO4, ETOH, DCM and H_20) and Time (1 hour) are Kept Constant

3.2 Central composite design model and data analysis

The central composite design is useful for establishing a mathematical model that relates the parameters of interest (such as temperature and extraction time) to the results obtained [23]. The CCD design consisting of 13 experimental runs was employed to investigate the optimization of natural dye extraction from *D. edulis* leaf using ethanol. It was observed from the experimental data (Table 2); that the yield obtained for 2 hours of extraction time at 65.9 ℃ was notably superior to that obtained with other experimental variables. A high value of this (0.8186) indicates a better extraction performance.

Table 2. The Independent Variables and their Corresponding Levels of Dye Extraction from *D. edulis* Leaf

Std	Run	Factor 1 A: Temperature (℃)	Factor 2 B: Time (Hr)	Response 1 Absorbance
9	1	80	2	0.6316
8	2	80	3.4	0.5338
12	3	80	2	0.6310
11	4	80	2	0.6296
5	5	65.9	2	0.8186
6	6	94.1	2	0.5216
4	7	90	3	0.4764
10	8	80	2	0.6316
7	9	80	0.6	0.5417

2	10	90	1	0.5200
3	11	70	3	0.7227
13	12	80	2	0.6319
1	13	70	1	0.6937

The statistical significance of CCD model was assessed using analysis of variance (ANOVA). The results of ANOVA, summarized in Table 3, indicate that the applied model was successful in navigating the design space. According to the data in Table 3, the correlation coefficient (R) has a high value of 0.9999, suggesting that over 99.08 % of the sample variables can be attributed to the variables in the model, with only 0.92 % of the total variance remaining unexplained. Adjusted R^2, which takes into account the sample size and several variables, is commonly used to evaluate the goodness of fit in models with multiple independent variables [24, 25]. In this case, the adjusted R^2 of 0.9999 is in close agreement with the predicted determination coefficient (Pred R^2 = 0.9998). It is worth noting that due to the large number of terms in the model and a relatively small sample size, the adjusted R^2 might be smaller [25]. The F-value and P-value also confirmed the significance of the model. A larger F-value and a smaller P-value indicate a more significant model. The obtained F-value of 25921.44 suggests the model's suitability and adequacy. Additionally, evaluating the residuals can provide insight into how well the model satisfies the assumptions of ANOVA [25, 26].

Table 3. Analysis of Variance (ANOVA) for the Response Surface Quadratic Model

Source	Sum of Squares	Df	Mean Square	F-value	P-value	
Model	0.1095	5	0.0219	25921.44	< 0.0001	Significant
A-Temperature	0.0882	1	0.0882	1.044E+05	< 0.0001	
B-Extraction time	0.0001	1	0.0001	98.31	< 0.0001	
AB	0.0013	1	0.0013	1560.20	< 0.0001	
A^2	0.0026	1	0.0026	3067.73	< 0.0001	
B^2	0.0153	1	0.0153	18099.41	< 0.0001	
Residual	5.912E-06	7	8.446E-07			
Lack of Fit	2.520E-06	3	8.400E-07	0.9906	0.4822	Not significant
Pure Error	3.392E-06	4	8.480E-07			
Cor Total	0.1095	12				

C.V. = 0.1496 %; R^2= 0.9999; Adjusted R^2= 0.9999; Predicted R^2=0.9998; Adeq Precision =546.2502

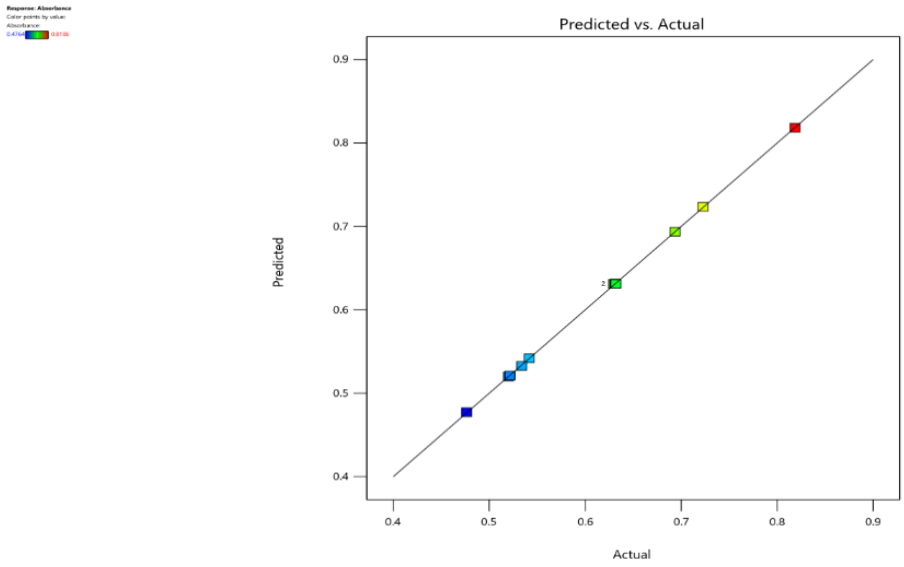


Figure 5. Correlation between actual experimental and predicted values

Figure 5 shows the predicted value for the response (outcome or result), the actual value or response. The diagnostic plots clearly demonstrate that the experimental values are in close proximity to the model predicted values. This observation suggests that the model is therefore a good fit for the experimental data. In support of these findings, a case study conducted by Hassani et al. [26] highlights the importance of diagnostic plots in data analysis. The study emphasizes the need to examine the proximity of data points to the regression line as an indicator of the model's performance. By incorporating the diagnostic plot, the researchers were able to validate the accuracy and suitability of their models for the experimental data.

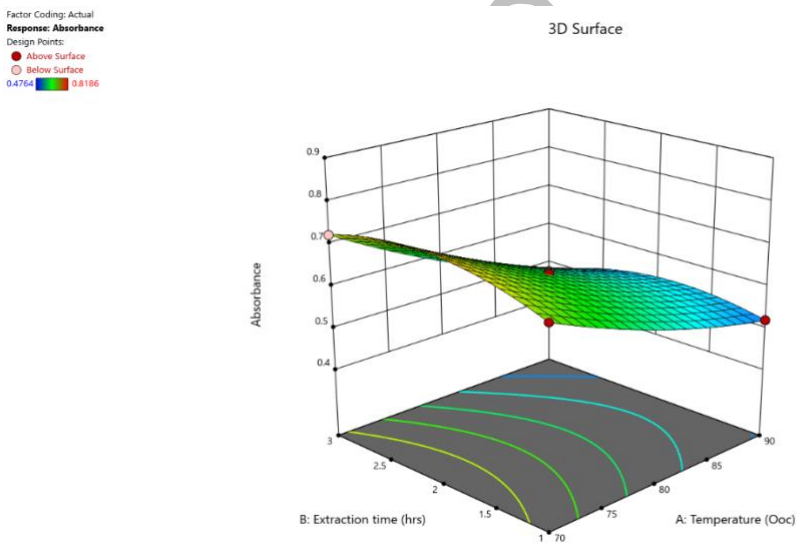


Figure 6. 3D plot for temperature and time interaction

The 3D response surface is commonly used in response surface methodology (RSM) to illustrate the interactive effects of factors such as temperature and extraction time on the response variable [27; 28]. Figure 6 presents a 3D plot that illustrates how temperature and extraction time interact to influence the response variable. The curved surface that peaks in the center and tapers off towards the edges suggests a strong interaction between two independent variables (extraction time and temperature), where the response variable reaches a maximum value

(0.8186) at the peak. By analyzing this plot, the optimal conditions for achieving the maximum dye extraction were observed at a temperature of 65.9 °C and an extraction time of 2 hours, as indicated in the 3D surface plots. However, exceeding an extraction time of 2-3.5 hours or a temperature range of 65.9 - 75 °C leads to a decrease in dye extraction rate which is consistent with the principle of thermodynamics and kinetics governing chemical reaction.

3.3 Phytochemical screening of the isolated dye

The process of extracting and conducting phytochemical screening on natural colourants from plant materials is a crucial step in identifying bioactive compounds. The crude dyes extracted from *D. edulis* leaves using a Soxhlet apparatus were analyzed, with the aim of identifying specific compounds. The crude extract obtained using ethanol was partitioned into thirteen fractions through the application of vacuum liquid chromatography [16]. Vacuum liquid chromatography (VLC) is a highly effective technique for the separation and purification of various compounds. The focus of this analysis was to identify key metabolites such as tannins, flavonoids and alkaloids, all of which are known for their diverse biological activities and dyeing potential. Upon screening, Fraction 13 which was the dye was found to contain metabolites such as tannins, flavonoids, terpenoids, steroids and alkaloids. However, cardiac glycosides were not detected. A comprehensive overview of the identified phytochemical groups in the fraction is provided in Table 4. It is noteworthy that these findings coincide with a prior investigation which also reported the presence of alkaloids, flavonoids, steroids, and terpenoids in the leaves of the same plant [29]. This consistency not only validates the reliability of the results but also supports the potential application of these compounds.

Table 4. Phytochemical Groups Present in *D. edulis* leaf Dye

Phytochemical group	VLC Fraction 13 (Isolated Dye)
Alkaloids	+
Steroids	+
Tannins	+
Flavonoids	+
Terpenoids	+
Cardiac Glycosides	-

Key: + Present, - Absent

Table 5. Constituents of the Isolated Dye from *D. edulis* Leaf

Compound	Phytochemical Group	Concentration (µg/ml)	Percentage composition Per Group
Benzoic acid (**1**)	Flavonoids	21.465	17.7
Isoquercetin (**2**)	Flavonoids	15.472	12.8
Rutin (**3**)	Flavonoids	19.563	16.1
Apigenin (**4**)	Flavonoids	17.250	14.2
Chlorogenic acid (**5**)	Polyphenols	47.500	39.2
Berberine (**6**)	Alkaloids	14.413	16.0
Vincristine (**7**)	Alkaloids	8.137	9.04
Vinblastine (**8**)	Alkaloids	10.165	11.3
Strictosidine (**9**)	Alkaloids	57.276	63.6
Tannic acid (**10**)	Tannins	40.082	100

Table 6: Colour Fastness to Light for *D. edulis* leaf

Ferrous sulfate Mordant			Control Unmordanted	
Sample code	Wool	Nylon 6	Wool	Nylon 6
	Colour Change	Colour Change	Colour Change	Colour Change
DA 2 %	5	4	3	2
DA 4 %	4	6	3-4	3
DA 6 %	6	5	5	3-4
DA 8 %	5	6	5	4

Key: DA-dye concentration, 1-very poor, 2-poor, 3-fair. 4-moderate. 5-good, 6-very good, 7-excellent, 8-outstanding

Table 7: Colour Fastness to Wash for *D. edulis* leaf

Ferrous sulfate Mordant			Control Unmordanted	
Sample code	Wool	Nylon 6	Wool	Nylon 6
	Colour Change	Colour Change	Colour Change	Colour Change
DA 2 %	4	2	2	2
DA 4 %	4	2-3	3	2
DA 6 %	3-4	4	3-4	3-4
DA 8 %	4	4	4	4

Key: DA-dye concentration, 1-poor, 2-fair, 3-good. 4-very good. 5-excellent

Table 8. Colour Fastness to Perspiration for *D. edulis* leaf

Ferrous sulfate Mordant					Control Unmordanted			
Sample code	Wool	Wool	Nylon 6	Nylon 6	Wool	Wool	Nylon 6	Nylon 6
	Acid	Akaline	Acid	Alkaline	Acid	Akaline	Acid	Alkaline
DA 2 %	3	2-3	3	4	2	2	2	2
DA 4 %	4	3	2-3	2	3	3	2	3
DA 6 %	3-4	4	3	3	2-3	4	3-4	4

DA 8 %	4	4-5	4-5	4-5	3	4	3	4

Key: DA-dye concentration, 1-poor, 2-fair, 3-good. 4-very good. 5-excellent

Table 9. Colour fastness to rubbing for *D.edulis* leaf

Ferrous sulfate Mordant					Control Unmordanted			
Sample Code	Wool	Wool	Nylon 6	Nylon 6	Wool	Wool	Nylon 6	Nylon 6
	Dry rubbing	Wet rubbing	Dry rubbing	Wet rubbing	Dry rubbing	Wet rubbing	Dry rubbing	Wet rubbing
DA 2 %	4-3	3	4	2	2	1	2	1
DA 4 %	3-4	3	4	3	2	1	3	2
DA 6 %	4-5	4	4-5	3	3-4	2-3	3-4	2
DA 8 %	5	4	5	4	4	3	4	3

Key: DA-dye concentration, 1-poor, 2-fair, 3-good. 4-very good. 5-excellent

Table 10. Colour coordinate values and colour strengths of wool and nylon 6 samples mordanted with $FeSO_4$ and dyed with dye isolate from *D.edulis* leaf

Sample	Concentration of mordant, %	K/S	L*	a*	b*	C*	H*
Non-mordanted wool		4.2	42.8	3.8	4.5	12.5	52.5
Iron Sulphate ($FeSO_4$)	2	6.7	32.5	5.6	7.6	15.6	53.2
	4	7.5	30.7	6.4	8.7	16.5	53.5
	6	8.6	28.8	7.5	9.2	18.6	53.8
	8	9.8	27.5	5.2	9.8	19.7	62.5
Non-mordanted nylon 6		4.0	34.7	3.7	4.6	11.6	56.2
Iron Sulphate ($FeSO_4$)	2	5.2	36.8	5.2	5.7	13.5	52.3
	4	6.3	34.5	5.6	7.6	15.2	51.3
	6	7.5	33.6	5.8	8.7	19.3	52.4
	8	8.7	30.7	7.0	9.5	20.4	57.6

Key: K/S- Strength of the shade of dyed sample, L* Brightness, a* Extent of redness, b* Extent of yellowness or blueness, H-Hue angle

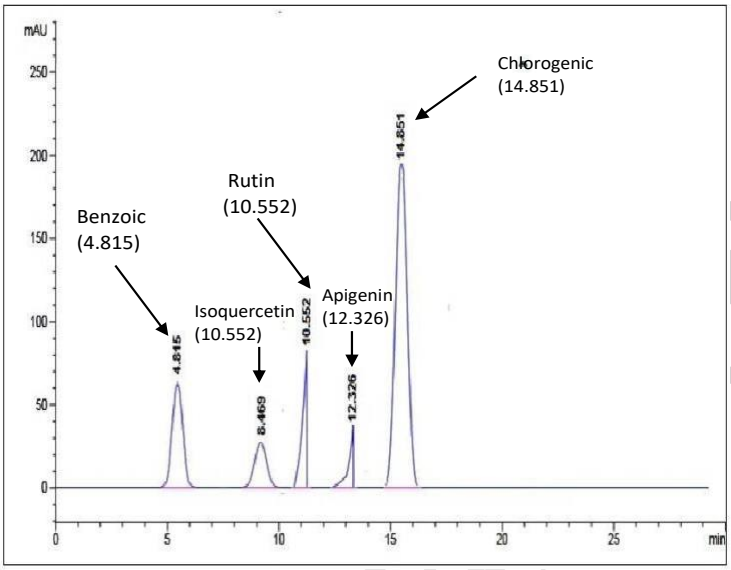


Figure 7. HPLC chromatogram of flavonoids and chlorogenic acid present in the isolated dye of *D.edulis* leaf

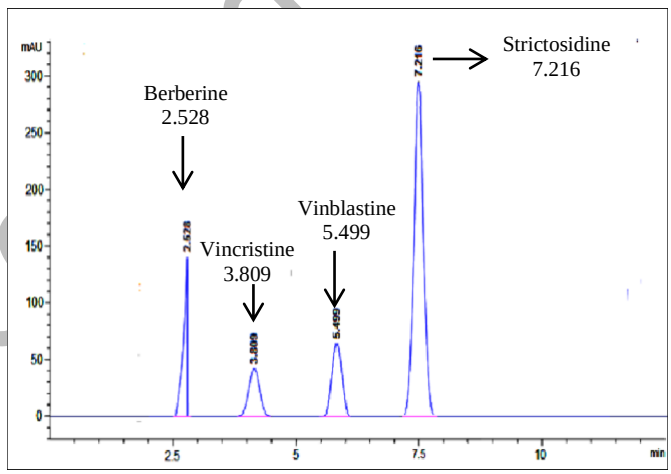


Figure 8. HPLC chromatogram of alkaloids present in the isolated dye of *D.edulis* leaf

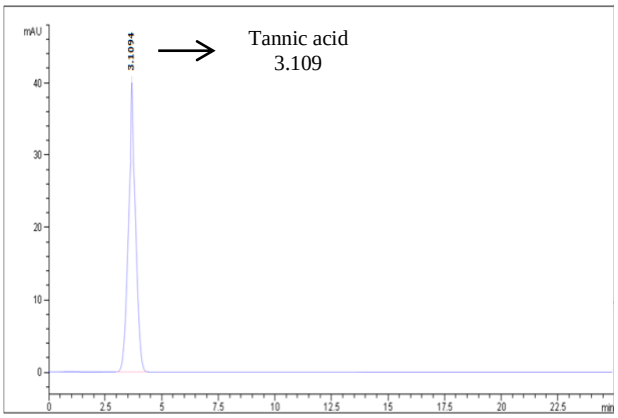


Figure 9. HPLC chromatogram of tannins present in the isolated dye of *D.edulis* leaf

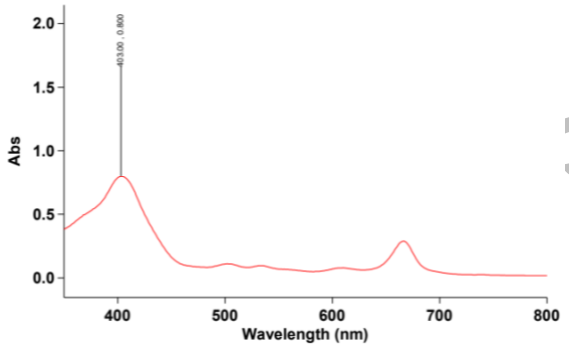


Figure 10. UV-visible spectrum of the isolated dye of *D.edulis* leaf

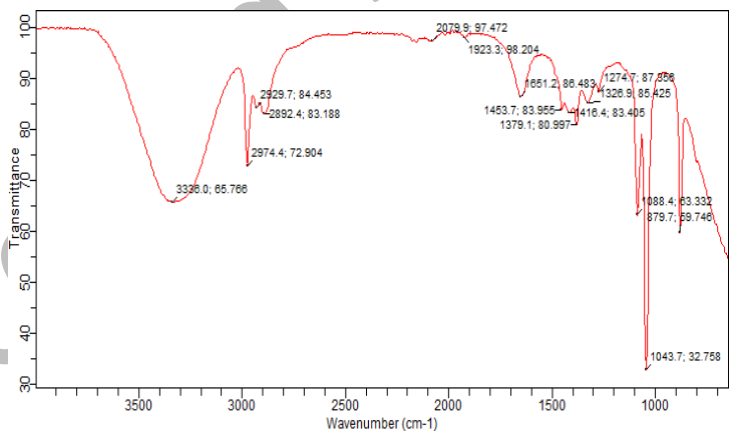


Figure 11. FTIR spectrum of the isolated dye of *D.edulis* leaf

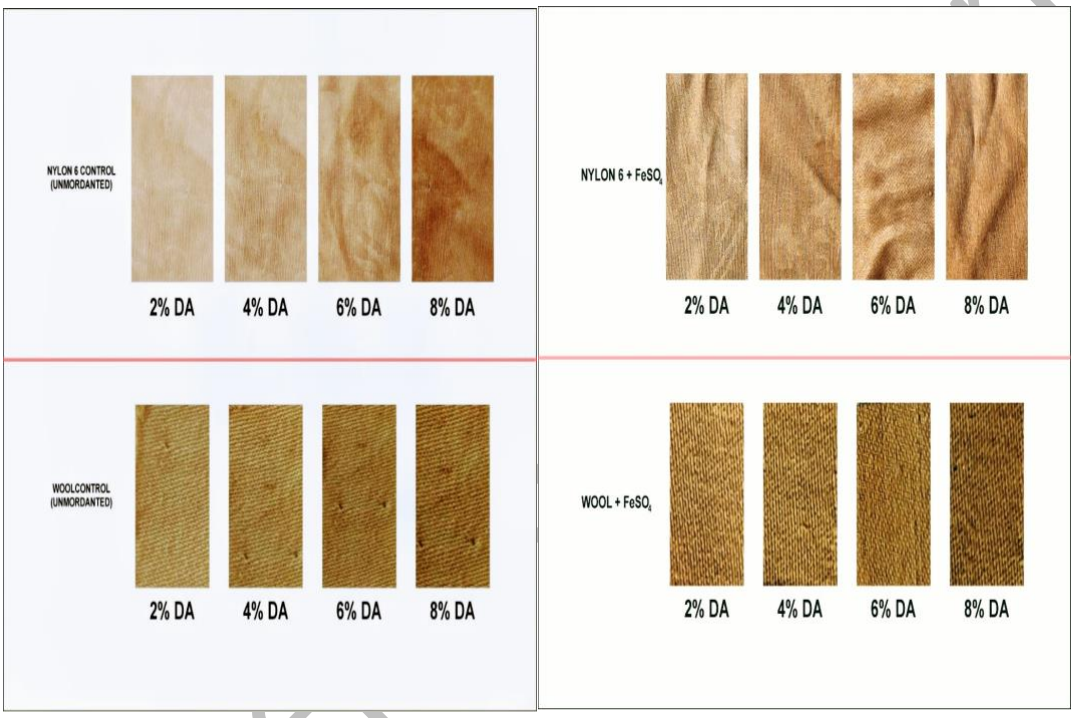
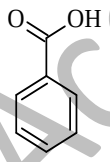


Figure 12. Scanned images of fabrics treated with and without mordant using dyes isolated from *Dacryodes edulis* leaf

3.4 Chemical composition of the colourant

Table 5 and Figure 7 indicate the identification of four flavonoids (apigenin, rutin, isoquercetin and benzoic acid) and chlorogenic acid present in the dye isolated from the leaves of *D. edulis*. Among these compounds, chlorogenic demonstrated the greatest affinity for the stationary phase, with a retention time of 14.851 minutes and a percentage composition of 39.2. Apigenin exhibited a longer retention time of 12.326 minutes, but a slightly lower percentage composition of 14.2 compared to rutin, which had values of 10.552 minutes and 16.1%. Isoquercetin had a retention time of 8.469 minutes and a lower percentage composition of 12.8 when compared to benzoic acid which had the lowest retention time of 4.815 minutes and a percentage composition 17.7. Table 5 and Figure 8 present the alkaloids present in the dye. Strictosidine was identified as the most abundant alkaloid, with a percentage composition of 63.6 and a retention time of 7.216 minutes. Vinblastine followed with a percentage composition of 11.3 and a retention time of 5.499 minutes. Vincristine had a percentage composition

of 9.04 and a retention time of 3.809 minutes. In contrast, berberine exhibited the shortest retention time and a comparatively high percentage composition among the alkaloids examined, with values of 2.528 minutes and 14.02% respectively. Table 5 and Figure 9 provide information on the tannin content of the dye. Tannic acid was the only compound detected for tannins, with a retention time of 3.109 minutes and a percentage composition of 100. Tannic acid is an organic compound with astringent properties. It is commonly used in the textile industry to enhance the colour fastness of dyes [30]. Berberine, vincristine and vinblastine are natural compounds derived from plants. Berberine is a traditional yellow natural dye that is biosynthesized from simple, primary metabolites such as acetate, isoprene and amino acids [31]. Vincristine and vinblastine, on the other hand, are native natural products that have been used in traditional medicine and have shown potential in modern medicine as well [32]. Strictosidine holds a significant role in the synthesis of a variety of biologically active compounds [33]. However, it is crucial to emphasize that the findings presented in this study cannot be directly compared to previous literature on *D. edulis* leaf. This is primarily due to the fact that this study represents the first attempt at the preliminary identification and quantification of these compounds. Furthermore, it is essential to acknowledge that retention times may exhibit variation depending on various factors such as solvent composition, extract matrix and the specific gradient elution program used. In previous studies conducted by other researchers, similar findings were reported regarding compounds like rutin, apigenin and benzoic acid. These compounds were identified and quantified through the use of high-performance liquid chromatography (HPLC). Their investigation discovered these compounds to be the principal colourant in their case study [34, 35]. Given the diverse biological properties and different chemical classes to which these compounds belong, they are viewed as a promising option for dyeing materials. Therefore, it is reasonable to suggest that the polar compounds observed in the dye isolated from *D. edulis* leaf may be accountable for its colouring potentials.



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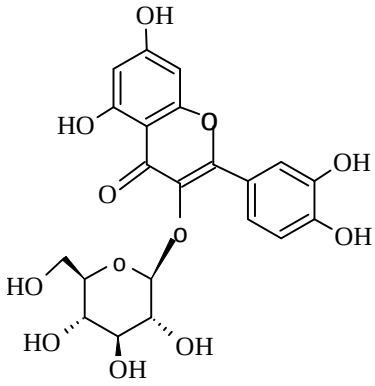

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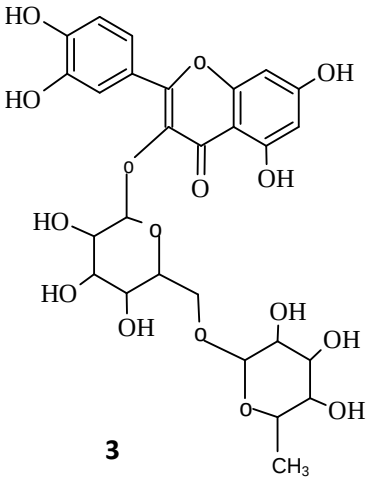

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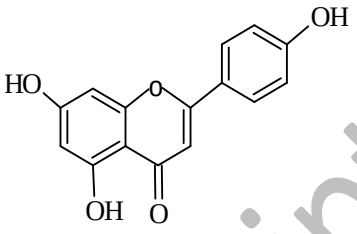

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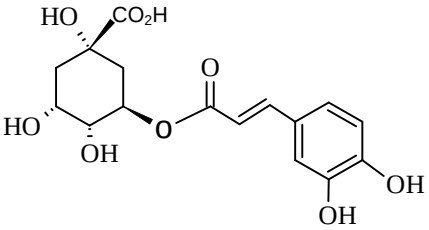

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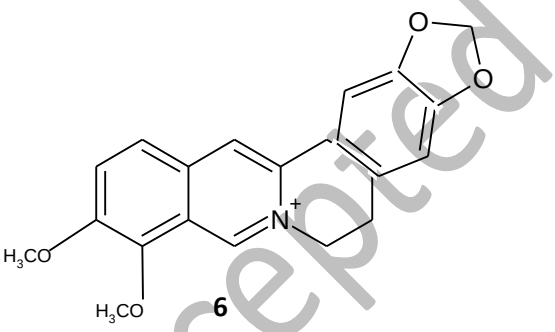

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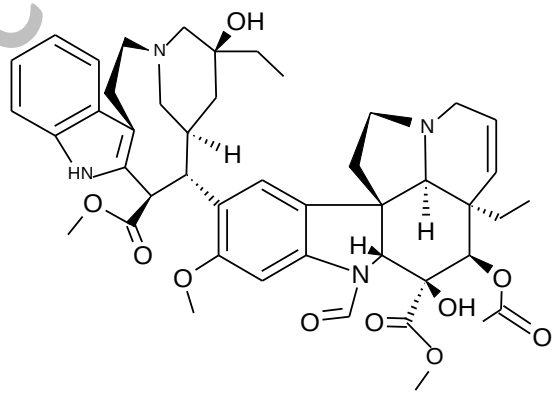

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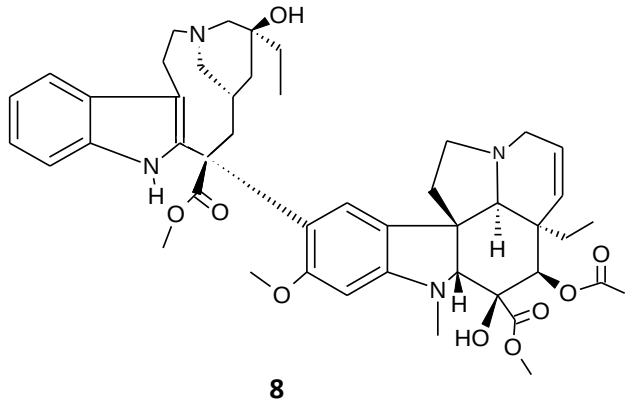

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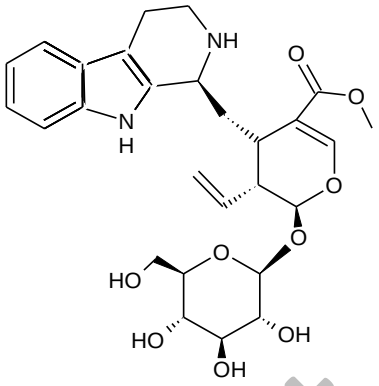

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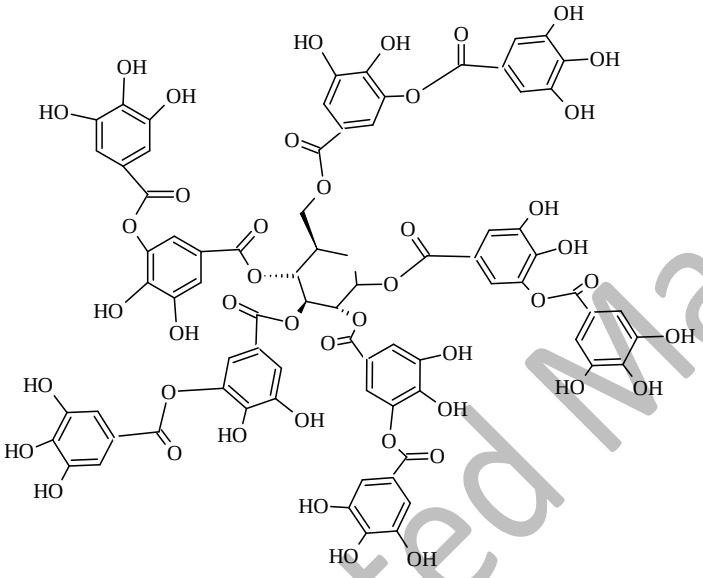

10

The ultraviolet-visible spectroscopy analysis of the dye obtained from *Dacryodes edulis* leaf was conducted within the wavelength range of 400-800 nm as shown in Figure 10. The maximum absorption peak was observed at 403 nm, which aligns with previous research that has established a correlation between the presence of alkaloids, and flavonoids to absorption peaks that range from 234 nm to 676 nm [36, 37]. Alkaloids are known to absorb light in the ultraviolet region of the spectrum of *Physalis minima*, specifically between 234 and 676 nm [36]. Similarly, flavonoids, a group of plant metabolites thought to provide health benefits through cell signaling pathways and antioxidant effects, also exhibit absorption maxima in this range [37]. Therefore, the presence of these secondary metabolites can be inferred from the absorption peak identified in this study within the aforementioned range. This finding supports the notion that *D. edulis* leaf is a rich source of these beneficial compounds.

The spectrum of the dye obtained from *Dacryodes edulis* leaf is in a frequency range of 3500-1000 cm^{-1} (Figure 11). A previous study reported that each spectrum displays a unique absorption band corresponding to a specific chemical composition of the dye [38]. Notably, the dye isolated from *D. edulis* leaf exhibited distinct bands within various segments of the spectrum: 3600-3200 cm^{-1}, 3100-2800 cm^{-1}, 1740-1640 cm^{-1}, 1650-1600 cm^{-1}, 1480-1300

cm^{-1} and 1300-900 cm^{-1}. These bands signify the stretching vibrations of different functional groups, such as O-H, C-H, C-C, C=C, C=O, CHO, aromatics, nitrile and amino acids. Several studies conducted by various researchers have extensively discussed and identified these functional groups corresponding to the various specific segments of the FT-IR spectrum [4, 39-41].

3.5 Colour fastness properties.

The ability of a material to maintain its colour characteristics and prevent the transfer of colour to adjacent white materials when in contact is known as colour fastness. Typically, colour fastness is evaluated through the use of a greyscale, either by assessing the loss of colour depth in the original sample or by examining any staining on nearby white material. However, among all types of colour fastness, light fastness and wash fastness are generally considered most important for any textile, while perspiration fastness is particularly relevant for apparel items [42].

3.6 Colour fastness to light

A colour fastness test was conducted on the dyed fabrics to assess their resistance to daylight (Table 6 and Figure and 10). The test results showed that the fabric samples denoted as DA 6 % (for wool), DA 4 % and DA 8% (for nylon) on mordanted samples registered a very good rating of 6 on the blue wool scale, while the unmordanted samples had fair to good ratings (3-5). This notable performance is likely due to the colouring component of the dye being an antioxidant, which is known to serve as a UV absorber, thereby enhancing the light fastness of naturally dyed fabrics [43]. These results are consistent with prior studies indicating that the lightfastness of dyed materials is significantly influenced by the characteristics of the compound substituents [44]. Additionally, factors such as symmetry and molecular size can contribute to the high resistance to light. When metal mordants are used, they can form coordination complexes with the functional groups of the natural dyes, further enhancing their light fastness. Moreover, the electronegative oxygen of the hydroxyl (OH) group within the dye structure such as quercetin interacts with the electropositive or cationic hydrogen (H) atom of the -NH-group in nylon 6, which leads to the formation of intermolecular hydrogen bonding between the H atom of the nylon 6 fabric and the electronegative oxygen (O) atom of the colouring compound.

According to research data, a fabric with a blue wool value above 5 is classified as having good resistance to sunlight exposure [45]. Therefore, the deviation observed in the test results for the aforementioned samples could be interpreted as an advantageous characteristic, pointing towards increased durability and longevity of these particular samples.

3.7 Colour fastness to washing

The wash fastness properties of the dyed fabric samples were evaluated and the results are presented in Table 7 and Figure 10. when nylon 6 and wool fabrics were dyed with *D. edulis* leaf, the washing fastness ratings were very good with a rating of 4 at a dye concentration of DA 8 % for both mordanted and unmordanted fabrics. A probable explanation for the good fastness properties is that tannin and flavonoids (isoquercetin and rutin) can form a metal complex with the ferrous sulfate mordant. Hence, after mordanting, the tannin and flavonoids are insoluble in water, thereby ultimately improving the washing fastness. These results suggest that the dyeing process using *D. edulis* leaf extract can be successfully applied to nylon 6 and wool fabrics due to their rich tannin content, which forms hydrogen bonds with carboxyl groups in the protein fibres like wool [46] and with amide group (-NH-) in nylon 6 fibres [47]. On the other hand, the ratings for nylon 6 fabrics, both mordanted and unmordanted showed the lowest rating at 2 and 2-3, for DA 2% and DA 4% respectively, indicating a minimal

colour change in the fabrics after washing. This implies that there was a lack of proper fixation of the dye on the nylon fabric, which is a critical step in the dyeing process [48]. Consequently, a larger number of dye molecules were lost during the washing process, leading to a poor rating on the scale.

3.8 Colour fastness to perspiration

The perspiration fastness of nylon and wool fabrics dyed with dye from *D. edulis* leaves were evaluated under acidic and alkali conditions (Table 8 and Figure 12). The fabrics that were mordanted with ferrous sulphate had very good to excellent fastness to alkali perspiration at 8% dye concentrations for both nylon and wool with a rating of 4-5. The fabrics also showed excellent fastness to acidic perspiration at 8% dye concentrations with a rating of 4-5 for mordanted fabric and good fastness to perspiration for unmordanted fabric with a rating value of 3. These results indicated that *D. edulis* dye is not only resistant to alkali perspiration but also to acidic perspiration. These results indicate that the alkali and acidic extract of *D. edulis* dye can produce fabrics that are resistant to perspiration in different environments. Furthermore, recent research by Prabhavathi et al. [49] supports the effectiveness of ferrous sulfate as a mordant in enhancing the perspiration fastness of natural dyes on textile materials.

3.9 Colour fastness to rubbing

The rubbing fastness results for nylon and wool at dye concentrations ranging from DA 2% to DA 8 % are presented in Table 9 and Figure 12. Comparatively, it is evident from the results that the dry rubbing performance for mordanted and unmordanted fabrics was superior to their wet rubbing performance for both wool and nylon 6, being rated as poor to very good (1-4). The difference in diffusion behavior between dyes with varying molecular weights and structures can be attributed to the low aqueous solubility of ferric-tannate complexes within the dyed fiber. When large molecular size complexes form in the dyeing bath, they have a very low diffusional behavior. As a result, these complexes primarily deposit on the periphery of the dyed fiber, leading to a low wet rubbing fastness. This phenomenon has been studied by Mongkholrattanasit et al. [48], whose research supports the idea that the diffusion behavior of these complexes is significantly influenced by their size and solubility characteristics. Further research indicates that dyes with larger molecular weights and intricate structures such as red 195, exhibit slower adsorption rates compared to those with lower molecular weights like blue 19 [50] affecting their diffusion dynamics during the dyeing process.

3.10 Colour measurement

The changes in K/S values of samples mordanted and unmordanted at various concentrations are shown in Table 10. The study found that the K/S values increased as the concentration of the mordants increased. This is in line with the results of the mordanted and mordanted samples, which showed deeper shades than those of the unmordanted samples. Additionally, the L values also decreased as the concentration of the mordant increased.

4. Conclusion

The study successfully extracted natural dyes from *Dacryodes edulis* and characterized the resulting dyes using various analytical techniques. It highlighted the significance of understanding the chemical composition and structure of these natural colourants, through methods such as UV-Vis spectrophotometry, high-performance

liquid chromatography (HPLC) and Fourier-transform infrared spectroscopy (FTIR). The extraction process was optimized to obtain high-quality colourant using ethanol at the optimal conditions of 65.9 ℃ for 2 hours. These findings provide valuable insights into the potential application of these natural colourants, particularly in textile fabrics like nylon 6 and wool. The isolated dye exhibited promising results, especially in terms of colour fastness to light ranging from moderate to very good, with a rating of 4-6 on the scale and somewhat fair to excellent levels of perspiration in both alkali and acidic conditions with a rating of 2-5. This study successfully demonstrated that the leaves of *Dacryodes edulis* can be effectively utilized for extracting a natural dye, presenting a sustainable solution with minimal negative environmental impact. This information is important for textile manufacturers seeking to adopt sustainable practices and reduce their reliance on synthetic dyes.

Conflict of Interest

The authors declare that they have no conflict of interest regarding the publication of this manuscript.

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