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Isolation and characterization of natural dyes from *Persea americana* leaves and their application on polyamide fabrics

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Abstract. This research investigated the extraction, isolation, and characterization of natural dyes from *Persea americana* leaves for application on polyamide fabrics. The solvent extraction process was optimized through Response Surface Methodology using a Central Composite Design (RSM-CCD), while techniques such as Vacuum Liquid Chromatography (VLC) and preparative High-Performance Liquid Chromatography (HPLC) were applied for dye isolation and purification. Characterization techniques like UV-Vis spectrophotometry, HPLC, FTIR, and NMR spectroscopy were used to identify the compounds responsible for the dyes' colouring properties. The study evaluated physical properties such as light fastness, wash fastness, perspiration fastness, and dry and wet rubbing fastness on polyamide fabrics. Optimal dye extraction conditions were found to be 55.6 ℃ over 3 hours. UV-Vis spectrophotometry revealed the presence of chromophores like conjugated systems in the dye fraction (VLC 13), while HPLC identified key compounds, such as quercetin and isoquercetin. FTIR spectroscopy detected functional groups typical of natural dyes, such as O-H, C-O-C, and C-O, while NMR spectroscopy confirmed the structures of two key constituents (quercetin and isoquercetin) of the dye. Mordanted fabrics showed deeper colour strength and improved fastness ratings ranging from fair to excellent (4-8 for light fastness, 2-4 for wash fastness and 2-5 for others). In contrast, unmordanted fabrics exhibited lower ratings (3-6 for light fastness, 2-4 for others, and 1-4 for rubbing fastness). These findings highlight the potential of utilizing *Persea americana* leaves, an often-underutilized agricultural by-product, to create bio-based textile treatments that promote green chemistry and sustainable manufacturing.

***Keywords*:** extraction; natural dyes; Response Surface Methodology; *Persea americana*; leaves; polyamide; fabrics.

1. Introduction

In recent years, there has been a growing interest in renewable and environmentally safe practices within the textile industry. This shift has led to increased research into natural dyes and compounds derived from plant sources as alternatives to synthetic dyes. Among the various plant species being explored, *Persea americana*, widely recognized as the avocado tree, has gained attention for its potential applications in textile dyeing due to its rich arrays of phytochemicals [1-4].

Persea americana, native to Central America and Mexico, is mainly grown for its fruit, which is called avocado, butter fruit, or alligator pear. Avocados are rich in essential nutrients, including unsaturated fatty acids, fiber, and vitamins B and E [5]. While the fruit is highly valued, recent research has turned its attention to the leaves of the avocado tree. These leaves are abundant in bioactive compounds such as flavonoids, phenolic acids, and tannins, which possess various beneficial properties [6]. Not only do these compounds exhibit potential dyeing abilities, but they also demonstrate antimicrobial, antioxidant, and UV-protective characteristics [7]. This makes them particularly appealing for innovative textile applications, where both functional and environmental improvements are sought.

Among several methods of extraction, Soxhlet extraction enhances the dyeing process by efficiently extracting natural dyes from plant materials through a continuous cycle of solvent washing. This method allows for the release of target compounds into the solvent, significantly improving extraction rates and yields. Interestingly, ferrous sulfate used as a mordant in natural dyeing plays a vital role in enhancing color fastness and can alter dye shades, often darkening the tones achieved [8].

The isolation and characterization of compounds from *Persea americana* leaves represent a significant step towards understanding their potential in textile application, particularly for polyamide fabrics. Nylon 6, also known as a synthetic polyamide, is a synthetic fiber widely used in the textile industry due to its durability, elasticity, and resistance to abrasion. However, it often lacks natural protective properties against microbes and UV radiation, which could potentially be addressed

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through the use of compounds extracted from *Persea americana* leaves. In contrast, wool is a natural, biodegradable, and renewable fiber recognized for its sustainability. It possesses inherent protective properties, such as natural UV resistance, moisture-wicking abilities, and antimicrobial characteristics. While wool is eco-friendly, there is an increasing need for synthetic fibers like nylon 6 to adopt similar sustainable attributes through bio-based materials like *Persea americana* leaves. This research aims to bridge the gap between the abundant natural resources provided by *Persea americana* and the rising interest in eco-conscious practices in the textile sector. By extracting, isolating and characterizing the compounds from avocado leaves and investigating their application on polyamide fabrics, this study contributes to the development of eco-friendly textile treatments that could potentially reduce the environmental footprint of textile production while enhancing the functional properties of the fabrics.

2. Experimental

2.1. Materials and reagents

In this study, *Pearsea americana* leaves were sourced from domestic waste in southern Nigeria. The plant specimen was verified and cataloged under voucher number 0614A at an established herbarium within the Federal University of Technology, Akure.

Analytical grade ferrous sulfate ($FeSO_4 \cdot 7H_2O$) served as the mordant, and the dye bath pH was adjusted to 7 by adding a 2 g/L sodium carbonate (Na_2CO_3) solution. A 5 g/L preparation of standard detergent A (ECE phosphate-free reference powder) was employed during the wash-fastness trials. All other reagents used in this investigation include concentrated 37% w/w HCl, *n*-hexane, acetonitrile, ethyl ethanoate, acetic acid (anhydrous), ethanol, Wagner's test solution (composed of iodine and potassium iodide), and sulfuric acid were of analytical grade and sourced from Merck (Darmstadt, Germany).

2.2. Processing of plant material

The leaves were gently rinsed with distilled water to eliminate dirt and surface impurities, ensuring the structural integrity of the plant material remained intact. The specimens were then subjected to extended shade-drying over several weeks, a preservation technique specifically chosen to maintain phytochemical stability [9]. After complete desiccation, the dried leaves were processed into a fine powder using a Porkert Manual Grinder (No. 32) and maintained at ambient temperature for later experimental analysis.

2.3. Process optimization for extraction

The pulverized powdered sample was subjected to preliminary extraction via Soxhlet apparatus using three different test solvents (ethanol (EtOH), dichloromethane (DCM), and distilled water) to determine the best solvent for the optimal yield of dye. For each extraction cycle, the extraction chamber housing a paper thimble containing 10 g of the powdered leaves was fitted to a round bottom flask containing 200 mL of the selected solvent and heated using a heating mantle. Extraction was conducted at six different temperatures (40 - 90 °C) for 1 h each, after thermal equilibration, spectrophotometric analysis was carried out on the crude extracts. Statistical optimization employed a Central Composite Design with five different levels for temperature (X_1) and extraction time (X_2) across 13 experimental runs in triplicate. ANOVA was performed using MODDE software ($p<0.05$). The statistically optimized variables were then applied in a second extraction [10], followed by fractionation of the crude extract using vacuum liquid chromatography (VLC).

2.4. Isolation

VLC was performed according to modified procedures adapted from the methods described by Paranagama and Ndukwe *et al.*, respectively [11, 12]. The column was vacuum-packed with TLC-grade silica to achieve compact and uniform packing. The ethanolic extract of *Persea americana* leaves was prepared for chromatography by mixing the extract with silica gel 70-230 mesh and gently loaded into the column. After the initial defatting, elution was performed in a stepwise manner using 300 mL each of suitable solvent mixtures. The polarity of the mobile phase was gradually increased by using mixtures of *n*-hexane and DCM in ratios of 3:1, 2:2, 1:3, followed by DCM, DCM-ethyl acetate blends (3:1, 2:2, 1:3), ethyl acetate and ethyl acetate-ethanol combinations (3:1, 2:2, 1:3), with the process concluding with ethanol. A total of 13 fractions were collected (VLC 1 – VLC 13). VLC 1 eluted with 100% *n*-hexane while VLC 13 eluted with 100% ethanol.

2.5. Qualitative phytochemical analysis

The dyes (VLC 1 and VLC 13) were analyzed using standard phytochemical procedures [12, 13] to verify the presence of secondary metabolites such as terpenoids, steroids, alkaloids, flavonoids, tannins, and glycosides.

2.6. UV-visible spectroscopic analysis

Spectrophotometric analysis of the dye (VLC 13) was conducted using a Shimadzu UV-3101PC spectrophotometer at room temperature with quartz cells and absorption changes in the 400 - 800 nm range were recorded. This was done to analyze the optical properties and determine the maximum absorbance (λ_{max}) of the isolated dye.

2.7. HPLC profiling

High-performance liquid chromatography (HPLC) analysis was performed on VLC 13 using an Agilent 1260 Infinity system, following a method slightly modified from [14]. A C18 reverse-phase column (250 mm × 4.6 mm, 5 µm) with a silica-based octadecylsilane stationary phase was used. For the detection of alkaloids, a binary solvent system consisting of 0.05 M ammonium acetate buffer (pH 5.0) and methanol was applied, with the detector set at 254 nm. Flavonoid profiling utilized a gradient elution of water and acetonitrile, each containing 0.1% formic acid, monitored at 365 nm. Tannins were analyzed using a mobile phase of water (0.1% trifluoroacetic acid) and methanol, with detection at 280 nm. The flow rate was consistently maintained at 1.0 mL/min, and the injection

volume was 20 µL across all analyses. Compound identification was based on comparisons of retention times of the dye components with authenticated standards.

2.8. Further spectroscopic analysis

Preparative HPLC using a Waters Prep-LC system isolated target compounds from VLC 13, which were analyzed via ^{1}H and ^{13}C NMR spectroscopy on a Bruker AV 600 to determine the structural features of the compounds of the isolated dye. Additionally, FTIR analysis was performed using a PerkinElmer spectrometer to detect functional groups in the isolated dye (VLC 13). A portion of the concentrated dye was directly placed on the diamond ATR crystal without additional treatment. The spectral data were collected within 3500 - 1000 cm^{-1} range.

2.9. Fabric pre-treatment

Wool and nylon 6 were chosen for this study because of their compatibility with natural dyes [8]. 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.

2.10. Mordanting

Pre-mordanting was applied to the pre-treated fabrics using 10 g of $FeSO_4 \cdot 7H_2O$ dissolved in 500 mL of distilled water to enhance dye absorption and fixation, in accordance with established methods [14]. A total of 8 pre-treated fabric samples (wool and nylon 6, each measuring 10 × 10 cm and weighing 2 g) were pre-soaked in warm water at 46 ℃ for 30 min to relax the fibers. The samples were then transferred to the mordant bath and treated at 46 ℃ for 45 min. After mordanting, the fabrics were conditioned at room temperature for 24 h, followed by washing and drying to remove excess mordant.

2.11. Dyeing procedure

The isolated dye (VLC 13) was applied to nylon 6 and wool fabrics according to the method described by Baka *et al.* [15]. Each dye bath's stock solution was prepared using different concentrations of VLC 13 and mordant. The volume required for each dye bath was calculated based on Equation 1:

$$V = W \times \frac{P}{C} \qquad (1)$$

where W = weight of fabrics, P = percentage shade, C = concentration of stock solution, V = volume, which is always unknown

2.12. Determination of wash fastness

Wash fastness was assessed according to ISO 105-C10:2006 [16]. Dyed 5 × 4 cm fabric samples were placed between two undyed pieces of the same size, stitched, and agitated with 10 steel balls in a 5 g/L soap and 2 g/L soda ash solution (liquor ratio 1:50) at 60 ± 2 ℃ for 30 minutes using a launder-o-meter. After rinsing and drying, color change and staining were evaluated using the ISO grey scale (ISO 9001:2000).

2.13. Determination of light fastness

Light fastness was evaluated using ISO 105-B01:2014 [17]. Fabric strips and blue wool standards were mounted on cardboard, half-covered to shield from light, and exposed to daylight at a 45° angle facing south for 72 hours. Fading was assessed by comparison with the blue wool scale.

2.14. Fastness to perspiration test

Perspiration fastness was conducted following ISO 105-E04 [18]. Two solutions were prepared: an acidic solution (5 g/L NaCl, 2.5 g/L $Na_2HPO_4 \cdot 2H_2O$) at pH 5.5, and an alkaline solution (0.5 g/L ε-aminocaproic acid hydrochloride, $C_6H_{14}ClNO_2 \cdot H_2O$) adjusted to pH 8 with 0.1 N NaOH. A liquor ratio of 20:1 was maintained during testing.

2.15. Determination of fastness to dry and wet rubbing

Rubbing fastness (dry and wet) was tested using a Crock meter in accordance with ISO 105-X12:2001 [19]. Standard white cotton cloth was rubbed against the dyed specimen under controlled pressure and speed for 20 strokes. The color transferred was assessed using the grayscale for color change (grades 1–5).

2.16. Measurement of dye uptake

Color strength of the dyed fabrics was assessed using a computer color matching system (CS-5), with reflectance at 420 nm measured by an ACS spectrophotometer. K/S values were calculated based on the Kubelka-Munk theory (Equation 2), which relates the absorption (K) and scattering (S) coefficients of a dyed substrate to its reflectance (R).

$$\frac{k}{s} = \frac{(1-R)^2}{2R} \qquad (2)$$

Higher K/S values indicate deeper or more intense coloration. The values were directly obtained for each sample using the same procedure.

3. Results and discussion

In this research, natural dye was isolated from *P. americana* leaves, characterized and applied to polyamide fabrics in the presence of a mordant; the durability of the resulting dyed fabrics was evaluated through multiple analytical and performance testing methods.

3.1. Maximum extraction yield

Optimization of process conditions for extraction, particularly temperature, is a critical factor in maximizing the yield of target compounds, a principle well-established in previous studies [14, 15]. This study examined Soxhlet extraction for *P. americana* leaves, with specific emphasis on temperature modulation within the range of 30 to 90 ℃ for 1 h each. The relationship between temperature variation and dye absorbance, as graphically represented in Figure 1, aligns with the trends observed in a similar study on another plant-based dye source [15].

Notably, ethanol demonstrated superior efficacy in dye extraction, achieving maximum absorbance of 0.58 AU at 80 ℃ compared to alternative solvents like dichloromethane (0.39 AU) and distilled water (0.33 AU) at their respective optimal conditions (Figure 1), a finding that corroborates the work of Al-Alwani *et al.* [20] on solvent efficacy in natural dye extraction. This observation supports the hypothesis proposed by Ghitescu *et al.* [21] which suggests that ethanol may enhance the release of compounds from plant tissues by disrupting their interactions, leading to more efficient extraction. The results of this study not only confirmed this theory but also demonstrated its relevance to *P. americana* leaves, an unexplored source of natural dyes. The slight decrease in absorbance at 90 ℃ for ethanol suggests that excessive heat may lead to compound degradation, emphasizing the importance of temperature optimization. The moderate performance of dichloromethane at lower temperatures (40-50 ℃) indicates that non-polar compounds may be more efficiently extracted under milder conditions, while the poor performance of water suggests that the target dye compounds in *P. americana* leaves are primarily lipophilic in nature.

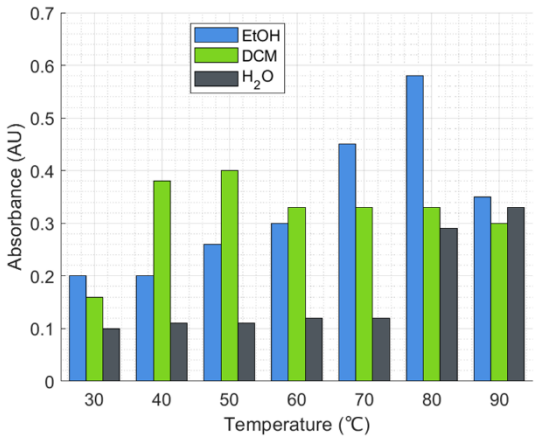

Figure 1. Effect of thermal parameter on *P. americana* leaves dye yield using various solvents with one-hour extraction time

3.2 Optimization modeling with CCD

The central composite design (CCD) is a powerful method for creating a mathematical model that connects critical parameters, like temperature and extraction time, to experimental results [22]. In this study, a CCD with 13 experimental runs was used to optimize the extraction of natural dye from *P. americana* leaves using ethanol. The experimental results (Table 1) indicated that an extraction time of 3 hours at 55.6 ℃ yielded the highest performance compared to other conditions tested, achieving a notable absorbance of 0.8250. This highlights the critical roles of temperature and extraction time in maximizing the extraction efficiency of *P. americana* leaves.

Moreover, these findings align with the research conducted by Rahman *et al.* [23], who discovered that temperature and extraction time had a substantial influence on the dye yield of *Xylocarpus moluccensis*. Similarly, Hidayati *et al.* [24] emphasized the importance of these variables in their study on the extraction of *Thevetia peruviana*, further reinforcing the understanding that optimizing these parameters is essential for achieving optimal extraction results. The results in Table 1 were subjected to various mathematical models to determine the best fit to the experimental result and these are shown in Tables 2-4.

Table 1. Levels of independent variables for dye extraction from *P.americana* leaves

Std	Run	Temperature (℃)	Time (h)	Absorbance (λ_{max} 435 nm)
2	1	90	3	0.500
7	2	80	2.6	0.5404
8	3	80	5.4	0.5200
12	4	80	4	0.6450
4	5	90	5	0.5100
11	6	80	4	0.6290
1	7	70	3	0.7250
5	8	55.6	3	0.8250
13	9	80	4	0.6250
3	10	70	5	0.6900
9	11	80	4	0.6400
6	12	94.1	4	0.5260
10	13	80	4	0.6260

The Central Composite Design (CCD) model was evaluated through Analysis of Variance (ANOVA), which helped determine the significance of the linear, quadratic and interactive effects [25]. The suitability of the quadratic polynomial model was confirmed using both the coefficient of determination (R^2) and ANOVA results.

Table 2. Variance analysis (ANOVA) for the quadratic response surface model

Source	Sum of Squares	Df	Mean Square	F-value	P-value	Remarks
Model	0.1101	5	0.0220	398.64	< 0.0001	Significant
A-Temperature	0.0857	1	0.0857	1550.33	< 0.0001	
B-Extraction time	0.0004	1	0.0004	6.56	0.0375	
AB	0.0005	1	0.0005	9.16	0.0192	
A^2	0.0034	1	0.0034	61.49	0.0001	
B^2	0.0178	1	0.0178	321.70	< 0.0001	
Residual	0.0004	7	0.0001			
Lack of Fit	0.0001	3	0.0000	0.2683	0.8458	Not significant
Pure Error	0.0003	4	0.0001			
Corrected total sum of squares	0.1105	12				

C.V. = 1.21%; R^2= 0.9965; Adjusted R^2 = 0.9940; Predicted R^2 = 0.9913; Adequate precision = 64.7504

According to Table 2, the model yielded an F-value of 398.64, implying that the model is highly significant, with only a 0.01% probability that this result is due to random variation. Factors A and B, their interaction (AB), and their quadratic terms (A^2 and B^2) demonstrated statistically significance at $p < 0.05$, while terms with p-values > 0.1000 were considered non-significant. This pattern points to a curved and complex response surface, which is often the objective in

optimization studies using RSM [15]. The lack-of-fit F-value was 0.27, indicating it is not significant, with just a 0.12% chance of occurring due to noise. The model's R^2 value of 0.9965 indicates that it accounts for 99.65% of the variability in the response with only 0.35% unexplained. Moreover, the adjusted R^2 (0.9940) and predicted R^2 (0.9913) were closely aligned. According to Chen and Qi [26], these values should ideally fall within 0.20 of each other to confirm a good model fit. The results of this study exceed this benchmark significantly, indicating an extraordinarily strong model fit.

Table 3 was used to check the suitability of different models.

Table 3. Summary of tested models for *Persea americana* leaves dye extraction

Source	Sequential P-value	Lack of Fit P-value	Adjusted R^2	Predicted R^2	
Linear	0.0005	0.0010	0.7340	0.5281	
2FI	0.6732	0.0008	0.7106	0.5272	
Quadratic	**< 0.0001**	**0.8458**	**0.9940**	**0.9913**	**Suggested**
Cubic	0.7520	0.6204	0.9925	0.9821	**Aliased**

As suggested by the software, the quadratic model was used for this study (Equation 3).

$$Y_{Dye\ Extraction} = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \beta_{ii} X_{ii} + \sum_{i<j}^{n} \beta_{ij} X_i X_j \ldots\ldots\ldots + \in \quad (3)$$

Table 4. Sequential sums of squares for *Persea americana* leaves dye extraction

Source	Sum of Squares	Df	Mean Square	F-value	P-value	
Mean vs Total	4.92	1	4.92			
Linear vs Mean	0.0860	2	0.0430	17.56	0.0005	
2FI vs Linear	0.0005	1	0.0005	0.1899	0.6732	
Quadratic vs 2FI	**0.0236**	**2**	**0.0118**	**213.57**	**< 0.0001**	**Sug-gested**
Cubic vs Quadratic	0.0000	2	0.0000	0.3019	0.7520	Aliased
Residual	0.0003	5	0.0001			
Total	5.04	13	0.3873			

Table 4 compares various models, including quadratic and two-factor interaction (2FI) models. The evaluation criteria include sum of squares (SS), mean square and Fischer value. The P-value obtained from Table 4 is remarkably less than 0.0001 (<0.0001), indicating a high level of statistical significance. This suggests that the quadratic model is significantly better at explaining the data compared to the 2FI model. The low P-values suggest a significant curvature in the response surface, indicating a significant relationship between these variables. These results strongly support the importance of the quadratic model in explaining the data. Additionally, Hassani *et al.* [27] conducted a comprehensive study on quadratic models and their effectiveness in explaining complex data sets. Their results agree with those of this analysis, which makes the drawn conclusion more valid.

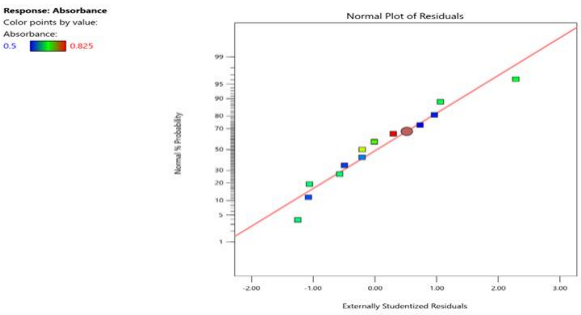

Figure 2. Validation plots (normal residuals plot) for dye extraction from *Persea americana* leaves

Figure 2 shows that the normal percentage probability and external studentized residual data plots exhibit a normal distribution. These plots are evenly distributed without any specific shape, suggesting that the model used is satisfactory. All data points fall within the predicted range, and the experimental values closely align with the predicted values of the model. The significance of normal distribution in statistical analysis has been extensively studied in scholarly articles. Frempong *et al.* [28] investigated the importance of normal probability plots in assessing the goodness of fit of statistical models. Their study emphasizes the need for evenly distributed data points, which aligns with the observations made in this analysis (Figure 2). Additionally, Mahfoudhi *et al.* [29] explored residual analysis in depth, highlighting the role of external studentized residual plots in model validation. Their findings validated the conclusions of the current investigation (Figure 2), as they stressed that a satisfactory model should exhibit a lack of discernible patterns or deviations in the residual plots.

3.3. Phytochemicals of the isolated dye

Vacuum liquid chromatography (VLC) effectively separated ethanolic crude extracts of *P. americana leaves* into 13 fractions (VLC 1 – VLC 13) based on the polarity and interaction of compounds with the silica gel. Maurya *et al.* [30], in their review of the isolation and purification of natural products, describe VLC as an effective technique for fractionation due to its ability to separate complex mixtures. Phytochemical screening was conducted on two active fractions (VLC 1 which eluted with 100% *n*-hexane and VLC 13 which eluted with 100% ethanol). This investigation sought to detect important secondary metabolites such as tannins, flavonoids, and alkaloids, recognized for their diverse biological functions and potential as natural dyes. The identified metabolites are detailed in Table 5. VLC 1 contained fewer metabolites compared to VLC 13, which contained key metabolites such as alkaloids, tannins, and flavonoids. This observation aligns with Jack *et al.* [13], who found that polar solvents often yield a broader range of phytochemicals in plant extracts. These results also agree with Boadi *et al.* [31], who detected tannins, flavonoids, terpenoids, steroids, and alkaloids in the leaves of *Persea americana*. Significantly, cardiac glycosides were not detected in either fraction, marking a notable negative finding in

phytochemical screening, contrary to Lawal *et al.* [1], who confirmed their presence. While traditional phytochemical studies focus on medicinal properties, this research investigates the phytochemical basis of plant-derived dyes, thereby contributing to the field of natural dye research.

Table 5. Metabolites of *P. americana* leaves dyes

Phytochemical group	VLC 13	VLC 1
Alkaloids	+	+
Cardiac Glycosides	-	-
Flavonoids	+	-
Steroids	+	+
Tannins	+	-
Terpenoids	+	+

KEY: + Present, - Absent

The main dye components of *P. americana* leaves were identified based on their retention time (Rt) and spectral characteristics (Table 6 and Figures 3-5). The isolated dye (VLC 13) from *P. americana* leaves contains four flavonoids (isoquercetin, quercetin, apigenin, kaempferol, luteolin), one phenolic compound (benzoic acid), five alkaloids (seneciphilline, echmidine, crinamine, crinamidine, strictosidine), and one tannin (catechins). Luteolin had the highest R-value (19.17 min) and abundance (12.4%) among flavonoids, while strictosidine had the highest R-value (8.93 min) and abundance (5.94%) among alkaloids. Isoquercetin had the lowest R-value (8.914 min) and lowest abundance (14.2%) among flavonoids, and seneciphylline had the lowest R-value (2.367 min) and lowest abundance (12.9%) among alkaloids. Catechins, the only tannin detected, had a retention time of 3.0791 min (100% abundance). Previous studies, including research by Park *et al.* [32], have investigated the therapeutic potential of compounds found in plant leaves and analyzed the chemical composition of *P. americana* leaves, identifying compounds like kaempferol. These findings highlight the diverse benefits derived from the chemical constituents present in the plant leaves.

Table 6. Compounds present in VLC 13 (isolated dye)

Compound	Concentration (µg/mL)	Relative percentage (%)
Phenolics:		
Benzoic acid	21.465	16.7
Flavonoids:		
Isoquercetin	15.472	12.0
Apigenin	19.859	15.5
Kaempferol	18.064	14.1
Quercetin	40.122	31.2
Luteolin	13.538	10.5
Alkaloids:		
Seneciphylline	10.938	12.9
Echimidine	4.464	5.25
Crinamine	5.643	6.63
Crinamidine	58.920	69.3
Strictosidine	5.048	5.94
Tannins:		
Catechins	20.784	100

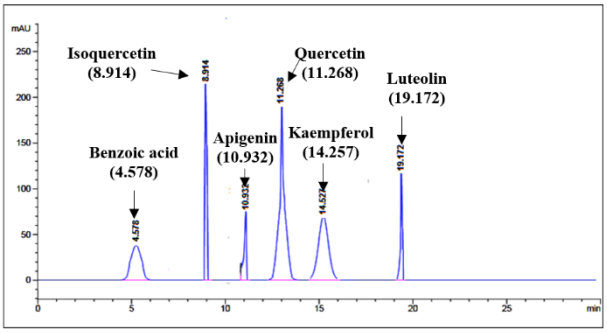

Figure 3. HPLC chromatogram of flavonoids content of the isolated dye (VLC 13)

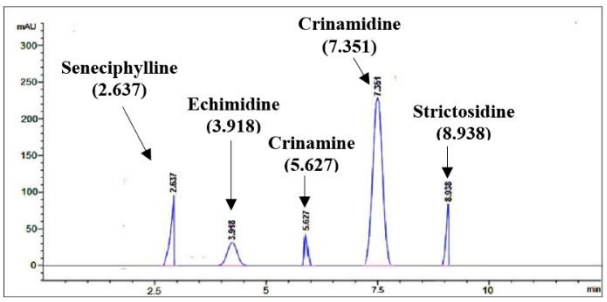

Figure 4. HPLC chromatogram of alkaloids content of the isolated dye (VLC 13)

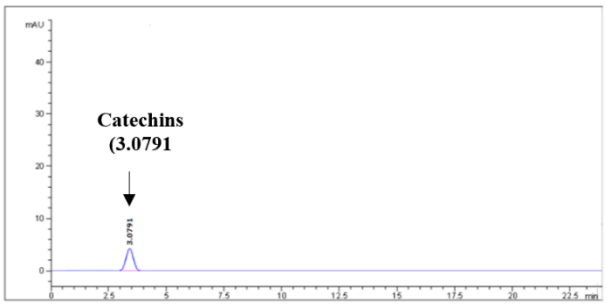

Figure 5. HPLC chromatogram of tannins content of the isolated dye (VLC 13)

The spectroscopic profile of VLC 13 (dye isolated from *Persea americana* leaves) was examined using UV-visible analysis (Figure 6). The spectrum exhibited an absorption peak at 409 nm, which is characteristic of $\pi \rightarrow \pi^*$ transitions, indicating the presence of many conjugated bonds in the isolated dye. This phenomenon has been extensively studied in various research papers and scholarly articles. Lojewski *et al.* [33] investigated the correlation between conjugated bonds and UV-visible spectra, highlighting the significance of these bonds in determining the observed wavelengths. Similarly, Zhao *et al.* [34] reported that the extent of conjugation in natural dyes directly correlates with their color intensity and fastness properties. Moreover, the specific wavelength of 409 nm falls within the violet-blue region of the visible spectrum, consistent with the findings of Narbona *et al.* [35], who observed that many plant-based dyes exhibit strong absorption in the blue-green region due to the presence of flavonoids and other polyphenolic compounds.

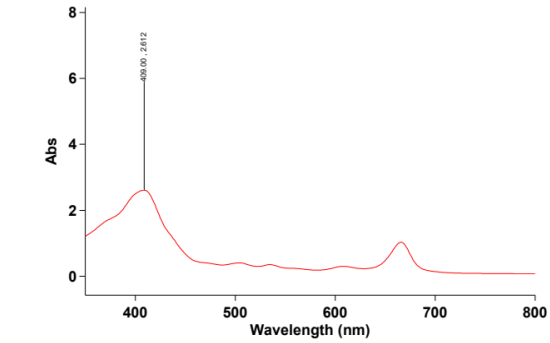

Figure 6. A UV-Vis spectrum of the isolated dye (VLC 13)

Notable absorption bands in the FTIR spectrum (Figure 7) of the isolated dye (VLC 13) were detected at 3324 cm^{-1} corresponding to O–H stretching vibrations, 2974 and 2885 cm^{-1} (C-H stretching), 1654 cm^{-1} (C=C stretching), 1453-1379 cm^{-1} (CH_3 bending), and 1043 and 1088 cm^{-1} (C-O stretching) match those found in other natural dyes. The O-H stretching band is linked to hydroxyl groups, as reported by Sofyan *et al.* [36]. The C-H stretching bands common in plant-based dyes were also identified by Talukdar [37]. The C=C stretching bands, indicating unsaturated bonds, align with the findings on *P. purpureum* leaves by Clark *et al.* [8]. These C=C stretching bands, often associated with aromatic structures, contribute to the colour-producing properties of natural dyes [38]. The C-O stretching typically suggests the presence of C-O-C and C-OH bonds, which may indicate carbohydrates, glycosidic linkages, and other oxygen-containing functional groups, all of which are believed to contribute to dyeing properties.

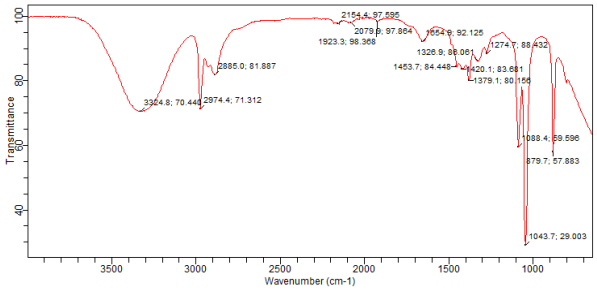

Figure 7. FTIR spectrum of the isolated dye (VLC 13)

In NMR spectroscopy, the chemical shift, expressed in parts per million (ppm) is an important parameter. This shift offers valuable insight into the electronic environment of carbon atoms within a molecule. In the case of quercetin, a polyphenolic compound derived from VLC 13, fifteen carbon signals were observed (205.2, 176.8, 148.1, 144.9, 142.0, 138.7, 136. 3, 134.9, 132.2, 128.2, 64.9, 62.9, 58.3, 53.1 and 53.1 ppm) (Figure 8). Specifically, the chemical shifts in the downfield range of 160-220 ppm indicate carbonyl carbon atoms that are deshielded by adjacent negative oxygen atoms while the mid-field region of 100-160 ppm encompasses signals from unsaturated carbon atoms present in alkenes, aromatics and other π bonds systems. The upfield signals in the 30-90 ppm range are characteristic of saturated carbon atoms connected to electronegative heteroatoms, aligning with the signals at 64.9, 62.9, 58.3, 53.1 and 53.1 ppm. These chemical shifts align well with the known structural characteristics of quercetin, which include a variety of hydroxy groups. These groups have a substantial impact on the electron density surrounding the carbon atoms, thereby influencing the observed chemical shifts [39].

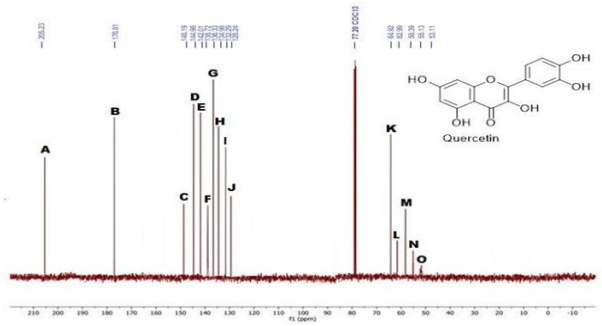

Figure 8. ^{13}C NMR spectrum of quercetin isolated from VLC 13

The ^{1}H NMR spectrum indicates aromatic protons showing doublet split signals (Figure 9). This splitting is typically a result of long-range coupling within the aromatic ring, a phenomenon that aligns with the chemical shifts of aryl protons found in the range of 6.5-8.0 ppm, which is considered the aromatic region [40]. The broad singlet encompassing the hydroxyl protons can be attributed to intramolecular hydrogen bonding with the carbonyl oxygen atom. This bonding leads to a distinct chemical shift and broadening of the signal, characteristics supported by studies on strong intramolecular hydrogen bonds which showed pronounced proton deshielding [41]. Considering the observed chemical shift and the proton coupling constants, and by comparing with the literature data, the compound isolated from VLC 13 (dye) have been identified to be quercetin [42].

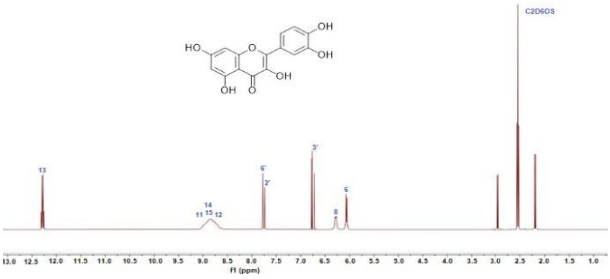

Figure 9. ^{1}H NMR spectrum of quercetin isolated from VLC 13

The NMR chemical shifts in of a second isolate from VLC 13 (Figures 10 and 11) were analyzed to be isoquercetin, a flavonoid compound known for its antioxidant properties. Isoquercetin, also known as quercetin-3-O-glucoside has a molecular formula of $C_{21}H_{20}O_{12}$.

A carbonyl carbon signal was observed in the ^{13}C NMR spectrum. In the structure of isoquercetin, this corresponds to the carbonyl group within the flavonoid core structure (Figure 10). Furthermore, the region from 148.1 ppm to 128.2 ppm contains multiple peaks that correspond to carbons within aromatic rings present in isoquercetin. The specific shifts can provide insights into the substitution pattern of the aromatic rings in isoquercetin [43]. Shifts observed at 64.9, 62.9 and 60.0

ppm are characteristics of oxygenated aliphatic carbons. In isoquercetin, these shifts are associated with the glucoside moiety, which contains multiple hydroxyl groups that would result in such shifts in the NMR spectrum.

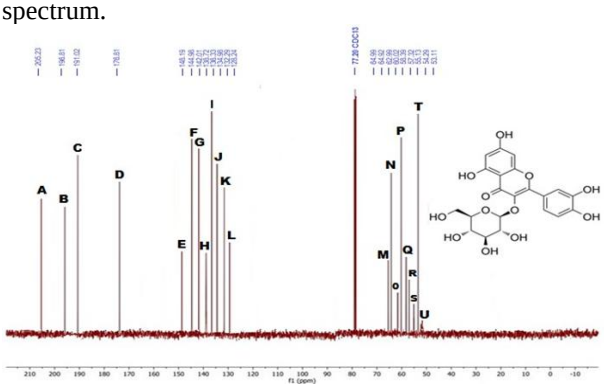

Figure 10. ^{13}C NMR spectrum of isoquercetin isolated VLC 13

^{1}H NMR spectrum of the isolated isoquercetin reveals distinct signals for the protons within both the flavonoid core and the glucose moiety (Figure 11). The aromatic protons of the flavonoid core appear within the 6.0-7.6 ppm range, which is consistent with the FT-IR spectral results suggesting the presence of aromatic rings. These observations further strengthen the findings from the UV-vis spectrum of the isolated dyes (VLC 13), where a strong absorption band in the 400 nm range due to the π-π* transition in aromatic rings is observed [33]. An anomeric proton, appearing as a doublet is detected at δ 4.52 ppm. Typically, the anomeric protons are attached to the carbon atom that is bonded to the oxygen atom of the glycosidic linkage. These chemical shifts confirm the structure of isoquercetin.

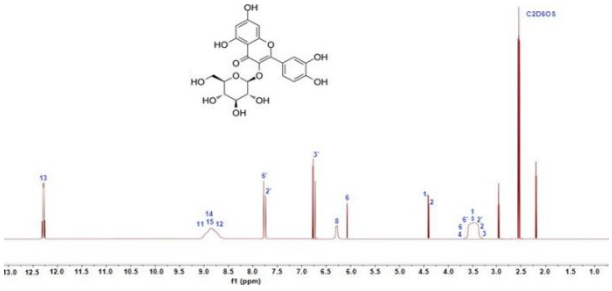

Figure 11. ^{13}C NMR spectrum of isoquercetin isolated from VLC 13

3.5. Color fastness properties

Color fastness properties measure how well a material maintains its coloration when subjected to external factors like light, rubbing, washing, and perspiration. These properties are crucial in evaluating the durability and quality of textiles. The colored fabrics were assessed for light fastness, wash fastness, perspiration, and wet & dry rubbing to evaluate their dyeing potential.

3.5.1. Light fastness. The test results in Table 7 and Figure 12 indicate that mordanted fabric samples, labelled as DC 6% (wool), DC 6% (nylon 6) and DC 8% (nylon 6) received a high rating of 6-7 on the blue wool scale, while unmordanted samples with other dye concentrations received lower ratings of 3-6. This variation is likely due to the presence of 3-hydroxy groups in quercetin, which can decrease light fastness by reducing photostability [44]. However, the presence of hydroxyl (O-H) or carbonyl (C=O) groups within the quercetin structure may improve dye-fiber bonding through hydrogen bonds, thereby enhancing color fastness. These bonds strengthen the adhesion between the dye molecule and the fabric (Figure 13), which is essential for textiles exposed to sunlight to maintain their appearance. The successful dyeing process may be attributed to the flavonoids present in the dye of *P. americana* leaves [31]. In addition, it was also observed that an increase in dye concentration correlates with improved photostability for both fabrics tested (Table 7), which is consistent with previous research [27]. The stability of a dye under light exposure is often related to the nature of its π - π* transitions. Dyes with stable π to π* transitions are less likely to degrade upon exposure to light, making them more suitable for applications where light fastness is important [45].

Table 7. Light fastness of VLC 13 (isolated dye)

	$FeSO_4$ (mordanted)		Control (unmordanted)	
Test code	Wool	Nylon 6	Wool	Nylon 6
DC 2%	4	4	3	4
DC 4%	5	5	4	4
DC 6%	7	6	5	4-5
DC 8%	8	6	6	6

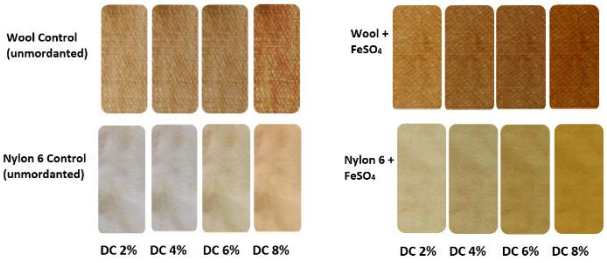

Figure 12. Scanned photographs of fabrics dyed with the isolated dye (VLC 13) comparing mordanted and non-mordanted samples

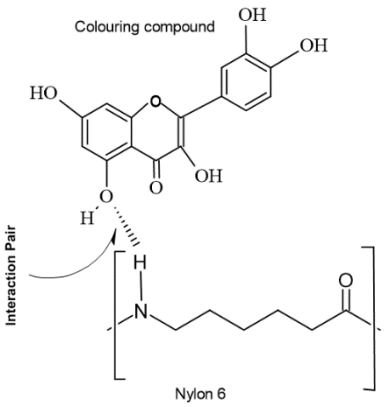

Figure 13. Proposed mechanism of dye adhesion with nylon 6 fabric

3.5.2. Wash fastness. The dyed fabric samples were evaluated for wash fastness, with results shown in Table 8 and Figure 12. When nylon 6 and wool fabrics were dyed with VLC 13, both mordanted and unmordanted samples displayed exceptionally good washing fastness ratings (4-5) at a dye concentration of 8% (DC 8%). Ratings for DC 2% - 6% are considered acceptable (2-4). Both nylon 6 and wool have complex molecular structures that can trap dye molecules within their

polymer chains. Wool, as a protein fiber, has an intricate structure that can tightly hold onto dye molecules. The excellent fastness properties observed, especially at higher dye concentrations, are likely due to strong interaction between the dye molecules from *P. americana* leaves and the fabric fibers (nylon 6 and wool). At higher dye concentrations (8% DC), dye molecules are more likely to penetrate deep into the fiber structure, making them more resistant to washing out. Although both mordanted and unmordanted samples showed good fastness, mordants typically enhance the bond strength between the dye and fiber. The impressive performance of unmordanted samples suggests that the dye from *P. americana* leaves have a natural affinity for these fibers. The leaves probably contain natural dyes with good substantivity (the ability to be absorbed and retained by the fiber).

Table 8. Wash fastness of VLC 13 (isolated dye)

	$FeSO_4$ (mordanted)		Control (unmordanted)	
Test code	Wool	Nylon 6	Wool	Nylon 6
DC 2%	4	2	2	2
DC 4%	4	2-3	3	2
DC 6%	3-4	4	3-4	3-4
DC 8%	4	4	4	4

3.5.3. Perspiration fastness. The perspiration fastness for nylon and wool fabrics dyed with VLC 13 tested under both acidic and alkaline conditions are given in Table 9 and Figure 12. The results revealed significant differences between mordanted and unmordanted fabrics. Fabrics treated with ferrous sulfate as a mordant showed very good to excellent fastness to alkaline perspiration at 8% dye concentrations, achieving ratings of 4-5 for wool and 3-4 for nylon 6. Wool fabrics mordanted with ferrous sulfate also exhibited excellent fastness to acidic perspiration, consistently achieving a high rating of 5, compared to the rating of 4 for nylon 6. In contrast, unmordanted fabrics had good perspiration fastness but with a lower rating of 3 for both wool and nylon 6. This contrast highlights the significant role of mordants in enhancing perspiration fastness, with mordanted fabrics (ratings 3-4 for nylon 6 and 4-5 for wool) outperforming unmordanted ones (ratings 3 for both wool and nylon 6). The findings suggest that *P. americana* dye can resists both alkaline and acidic perspiration, with improved stability across different pH levels when mordanted. This indicates that the ferrous sulfate-dye-fiber complex is resistant to pH changes typical of perspiration. These observations align with existing literature; that mordanting with metal salts improves the pH stability of natural dyes on wool fibers and protein fibers generally exhibit strong affinity and fastness properties with plant-based dyes due to their unique chemical structures and functional groups [46, 47].

Table 9. Perspiration fastness of VLC 13 (isolated dye)

	$FeSO_4$ (mordanted)				Control (unmordanted)			
Test code	Wool acid	Wool alkaline	Nylon 6 acid	Nylon 6 alkaline	Wool Acid	Wool alkaline	Nylon 6 acid	Nylon 6 alkaline
DC 2%	2-3	4	4	3	2	2	2	3
DC 4%	4	4-3	4	3-4	2	2-3	2	4
DC 6%	3-4	4	3-4	4	2-3	4	3	4
DC 8%	5	4-5	4	3-4	3	4	3	4

3.5.4. Rubbing fastness. Table 10 and Figure 14 show the rubbing fastness results for nylon 6 and wool with concentrations between 2% to 8%. Samples without mordants exhibited lower rubbing fastness, with ratings between 1-4, compared to mordanted samples, which were rated from 2-5. This difference is attributed to mordants, which enhance dye fixation by forming stronger chemical bonds with the fibers, thereby securing the dye more effectively. Unmordanted samples, on the other hand, have weaker dye attachments and are more susceptible to color loss during rubbing. Additionally, these unmordanted fibers tend to have rougher surfaces or uneven dye distribution, which further reduces fastness. Mordants also improve abrasion resistance by smoothing the fiber surfaces and enabling deeper dye penetration. Dry rubbing fastness showed better performance with ratings from fair to excellent (2-5) than wet rubbing fastness which had a rating of 1-4, likely because moisture weakens the dye-fiber bonds, making dye transfer easier under wet conditions. Both wool and nylon 6 also absorb moisture and swell when wet, causing the fiber structures to expand, which facilitates easier dye migration to the surface during rubbing.

Table 10. Rubbing fastness of VLC 13 (isolated dye)

	$FeSO_4$ (mordanted)				Control (unmordanted)			
Test 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
DC 2%	3-4	2	2-3	1	2-3	1	2	2
DC 4%	4	3	4	2-3	2	1	3	2
DC 6%	4	3-4	5	3	3-4	3	3-4	2-3
DC 8%	4-5	4	5	3-4	4	3	4	3

3.6. Color measurement

The variations in absorption coefficient to scattering coefficient ratio values for both mordanted and unmordanted samples across different concentrations are given in Table 11. The investigation revealed a positive correlation between mordant concentration and K/S values. This finding is consistent with the observed color intensity, where mordanted specimens exhibited richer hues compared to their unmordanted counterparts. Furthermore, an inverse relationship was noted between mordant concentration and L values, with higher concentrations resulting in lower L values. These results concede with previous research which examined dyeing silk with eucalyptus leaf extract and found that increasing mordant concentration improved K/S values [48]. This consistency highlights the similar effects of mordants in various natural dyeing methods.

Table 11. L*a*b* C*H* values for VLC 13 (isolated dye)

Sample	Concentration of mordant (%)	K/S	L*	a*	b*	C*	H*
Non-mordanted wool		3.2	46.7	3.7	5.2	13.6	53.6
$FeSO_4$	2	5.4	34.5	5.8	7.8	15.5	54.2
	4	6.8	33.3	6.7	8.3	16.6	54.5
	6	8.6	32.4	7.6	9.7	18.7	54.8
	8	9.7	30.2	5.6	9.6	19.8	62.4
Non-mordanted nylon 6		4.2	35.6	3.8	5.6	14.5	57.4
$FeSO_4$	2	4.8	37.2	5.4	4.8	13.7	54.5
	4	6.3	34.6	5.7	8.4	15.3	51.8
	6	7.5	33.8	6.3	8.6	18.4	53.5
	8	8.6	31.5	7.6	9.7	20.8	58.6

4. Conclusions

The study demonstrated the successful separation and characterization of plant-based dyes from *Persea americana* leaves. The optimized extraction method, using ethanol under specific conditions, yielded high-quality dyes suitable for textile applications. The dye showed superior performance in terms of color fastness and resistance to perspiration, while the incorporation of fixative substances significantly enhanced their overall dyeing effectiveness, making it a viable eco-friendly alternative to synthetic dyes, and offering sustainable options for the textile industry.

Conflict of interest

The contributors to this study confirm the absence of any competing interests related to the published findings.

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