

Optimization of Eco-Friendly Mordant-Assisted Extraction and Stability of Natural Pigments from Six Plant Species for Pressed Paint Development

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Abstract

This study aimed to optimize the decoction extraction efficiency of natural pigments from 6 plant species—*Clitoria ternatea* L., *Scleropyrum pentandrum*, *Vitex pinnata* L., *Oroxylum indicum* L., *Xylia xylocarpa*, and *Careya sphaerica*—by systematically varying extraction temperature (60 and 80 °C), time (30 - 90 min), and extraction conditions (normal and mordant-assisted). The main objective was to improve pigment yield and stability for potential use in sustainable natural dye and colorant applications. Results indicated that higher extraction temperatures (80 °C) and extended extraction times (90 min) significantly increased pigment yields, with *Clitoria ternatea* L. achieving the highest yield (12.92 % ± 0.03) under standard conditions. In contrast, the condition at 60 °C has a % yield of 11.47 ± 0.02. Mordant-assisted extractions increased overall yields (3.16 - 4.57 %) but required careful parameter optimization to avoid degradation of thermolabile compounds. UV-vis spectroscopy revealed that both extraction conditions and sodium chloride addition as a mordant significantly enhanced pigment absorption, particularly in *Oroxylum indicum* L., which showed optimal light absorption at 60 °C for 60 min (4.16 ± 0.09). Cytotoxicity assays demonstrated that most extracts exhibited minimal toxicity at low concentrations (< 0.1 mg/mL), with *Oroxylum indicum* L. showing the highest cell viability (95.65 ± 1.32 %). Pressed paint products formulated with sodium chloride-mordanted pigments displayed good stability under various conditions. The optimal extraction conditions identified in this experiment are as follows: the best temperature is 80 °C, the optimal extraction time is 90 min, and the use of a mordant is necessary for all plant species tested. Among the plants studied, *Clitoria ternatea* L. yielded the most intense color with a dark blue shade and the lowest L* value (38.14), indicating the highest color intensity under these conditions.

Keywords: Decoction extraction, Natural pigments, Mordant, Sodium chloride, Cytotoxicity, Pressed paint products

Introduction

Humanity has long utilized natural materials to dye fibers and fabrics for clothing and daily use. Natural pigments, valued for their color and potential health benefits, are increasingly applied across industries such as food, pharmacology, toxicology, textiles, printing, dairy, and fisheries due to their cost-effectiveness and

environmental advantages [1]. To promote sustainability and affordability, research should focus on developing pigments that are easy to use, non-toxic, eco-friendly, inexpensive, and biodegradable [2]. Natural dyes are classified by source into mineral dyes—derived from metal compounds like copper, iron, and

chromium, historically significant but now less used; animal dyes-obtained from insect secretions or marine animals [3], such as lac in Thailand for bright red hues; and plant dyes, the primary category, which produce a wide spectrum of colors. Plant-based reds come from sources like madder root and sappanwood; yellows from turmeric and ginger rhizomes [4]; browns from various lamduan barks [5]; blues from indigo leaves; and blacks from fruits like Malabar nut, reflecting a rich diversity of natural colorants traditionally employed in Thai culture and beyond.

Additionally, there are 6 types of plants that provide interesting colors, including *Clitoria ternatea* L., *Scleropyrum pentandrum*, *Vitex pinnata* L., *Oroxylum indicum* L., *Xylia xylocarpa*, and *Careya sphaerica* were selected for dye extraction because they are locally available, possess high pigment content and bioactive compounds, and have demonstrated potential for producing natural dyes with good color properties and additional benefits such as antimicrobial or anti-inflammatory activity, making them suitable and sustainable sources for natural dyeing [6-8]. In terms of natural pigment extraction from plants is crucial for various industries, including textiles, food, cosmetics, and pharmaceuticals. Traditional methods include decoction, maceration, infusion, and Soxhlet extraction, while modern techniques like microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE) are used. Decoction involves boiling plant materials, particularly roots, barks, and seeds, to extract water-soluble and heat-stable compounds. It is effective for extracting fibrous plant materials and produces concentrated extracts with higher potency [9]. Dyeing methods are typically classified into cold dyeing, which uses natural plant materials, and hot dyeing, which involves heating plant matter [10]. To improve dye adherence and color brightness, chemical mordants-mainly metal salts such as alum [11,12], copper, and iron-are commonly added [13,14]. Alum enhances reds and yellows, copper deepens greens and browns, and iron darkens shades or alters colors. These mordants form coordination complexes with both dye and fiber, stabilizing the color and improving fastness. For example, aluminum ions bond with hydroxyl or carboxyl groups on fibers and dyes, while copper ions coordinate with amino or hydroxyl groups, especially in protein-based or cellulose fibers. Although commercial

mordants are effective, overuse can leave residues and damage fibers, particularly with iron. Natural mordants-including lime water, lye, plant acids, and rust water-can also fix and sometimes darken colors, offering a more environmentally friendly alternative. Salt has been shown to act as a natural mordant, enhancing color intensity, especially in eco-printing [15]. Because most natural dyes do not naturally adhere well to fibers, especially cellulose [16], mordants are essential for bridging dye molecules to fibers [17], creating a range of colors and improving colorfastness [18]. Recent research focuses on natural mordants to address environmental concerns associated with heavy metal mordants [19-22].

The research team selected 6 local Thai plants-*Clitoria ternatea* L., *Scleropyrum pentandrum*, *Vitex pinnata* L., *Oroxylum indicum* L., *Xylia xylocarpa*, and *Careya sphaerica*-for their natural pigments and traditional use in earth-tone dyeing. Each plant contributes distinct hues: *Clitoria ternatea* L. yields deep blues and purples, *Scleropyrum pentandrum* provides greenish-olive tones, *Vitex pinnata* L. offers yellow to brown shades, *Oroxylum indicum* L. and *Xylia xylocarpa* supply rich browns, and *Careya sphaerica* produces yellow to brown pigments. These plants were chosen for their sustainability, non-toxicity, local availability, and cultural relevance in Thai textile crafts. Dye extraction uses the decoction method, varying temperature, time, and mordant type, followed by testing for color properties and toxicity. Optimal conditions are then used to develop pressed paint products for art and potential industrial applications.

Materials and methods

Materials and equipment

The plant-using test includes *Clitoria ternatea* L., *Scleropyrum pentandrum*, *Vitex pinnata* L., *Oroxylum indicum* L., *Xylia xylocarpa*, and *Careya sphaerica* purchased from Thai herb commercials in Thailand. The materials purchased from sChemipan, Thailand, include sodium chloride, magnesium stearate, silicon dioxide, phenoxyethanol (and) chlorphenesin (and) glycerin, and cornstarch. The toxicity test used sodium lauryl sulfate (SLS) was a standard substance, which involved human skin fibroblast cells from passage 66, and the cell culture medium was Dulbecco's modified Eagle medium (DMEM) with 10 % fetal bovine serum (FBS) and 1 %

penicillin/streptomycin. The equipment used in the experiment included the extraction using a magnetic hotplate stirrer (IKA, C-MAG HS7, Germany), evaporation using a rotary evaporator (BUCHI, R-300, Switzerland), the toxicity test using equipment including a microplate reader (BMG Labtech, SPECTROstar Nano, Germany) and Laminar Flow Cabinet (Thermo Scientific, HERA Guard Eco 1.2, Germany).

Extraction of color from plants

The extraction of natural colorants from 6 plant sources-*Clitoria ternatea* L. (flower), *Scleropyrum pentandrum* (heartwood), *Vitex pinnata* L. (heartwood), *Oroxylum indicum* L. (bark), *Xylia xylocarpa* (bark), and *Careya sphaerica* (bark)-involves a systematic process of washing, oven-drying at 50 °C for 48 h [23], size reduction, and decoction extraction under varied temperature-time parameters (60 - 80 °C for 30 - 90 min) as shown in **Table 1**. The extraction process concludes

with filtration through Whatman No. 1 paper and concentration via rotary evaporation, with experiments conducted in triplicate for statistical validity. Research indicates decoction is superior for antioxidant extraction compared to methods such as maceration and sonication, with extraction efficiency significantly influenced by temperature and duration parameters [24]. Studies demonstrate that optimal extraction conditions vary by plant material, with 44 °C for 93 min maximizing anthocyanin yield, while heat-sensitive plants benefit from temperatures of 60 - 80 °C for 90 - 120 min [25]. Additionally, mordants-particularly NaCl at concentrations of 10 % w/v-play a crucial role in enhancing color fastness, depth, and brightness when applied to cotton fabrics, either independently or in combination with metal mordants such as CuSO₄ and FeSO₄. Various extraction parameters directly affect pigment yield and color characteristics, as evidenced in studies of black tea waste extraction at 80 °C across different time intervals [26,27].

Table 1 Extraction conditions for 6 plant types using decoction: Temperature, time, and parameter variations.

Condition	Temperature (°C)	Time (min)	Parameter
1	60	30	Normal
2	60	60	Normal
3	60	90	Normal
4	80	30	Normal
5	80	60	Normal
6	80	90	Normal
7	60	30	Mordant (10 % w/w NaCl)
8	60	60	Mordant (10 % w/w NaCl)
9	60	90	Mordant (10 % w/w NaCl)
10	80	30	Mordant (10 % w/w NaCl)
11	80	60	Mordant (10 % w/w NaCl)
12	80	90	Mordant (10 % w/w NaCl)

Light absorption measurement using UV-Visible spectroscopy method

The experiment involved scanning the solutions of six types of plants, including *Clitoria ternatea* L., *Scleropyrum pentandrum*, *Vitex pinnata* L., *Oroxylum indicum* L., *Xylia xylocarpa*, and *Careya sphaerica*, to find the wavelength that absorbs the maximum light (λ_{max}) using a UV spectrophotometer (JASCO, V-730, Japan) in the wavelength range of 200 - 1,000 nm. Once

the maximum light wavelength (λ_{max}) for each type of plant was obtained, the amount of light absorbed was measured, compared, and recorded. Previous research determined λ_{max} by scanning the wavelength range, starting from preparing the sample solution and then scanning the absorbance in the predicted wavelength range (200 - 1,000 nm) to find the λ_{max} that gives the highest absorbance [28].

Color measurement method

The color measurement protocol employs a Hunter LAB Miniscan XE plus colorimeter (USA) to analyze plant extract color values under various conditions. Prior to each measurement, the device is calibrated using a standard white plate ($L^* = 92.65$, $a^* = -0.82$, $b^* = 1.31$) to ensure accuracy. Color assessment utilizes the CIE Lab* system, which measures color through 3 dimensions: L^* (lightness), a^* (red-green spectrum), and b^* (yellow-blue spectrum), under standardized D65 Observer 10 ° lighting conditions (6,500 K daylight, 10 ° viewing angle). Each measurement is conducted in

triplicate to minimize variability and enhance result reliability, with values recorded after completion.

Pressed paint product method

The pressed paint product development process involves selecting optimal plant-specific conditions using ingredients shown in **Table 2**. The production begins by thoroughly mixing Phase A ingredients before incorporating Phase B until well combined. The combined mixture is then prepared for molding by weighing 10 g of pigment powder into a 54 mm pan. Finally, the product is formed using a mold machine operating at 150 bars of pressure to create the finished pressed paint product.

Table 2 The formulation of pressed paint product.

Phase	Ingredient	% w/w	Function
A	Extract of plant	23	Coloring agent
A	Silicon dioxide	3	Absorbent agent
B	Magnesium stearate	8	Binder
B	Corn starch	65	Texture
B	Phenoxyethanol (and) chlorphenesin (and) glycerin	1	Preservative

Drop test

The drop test was conducted to evaluate the durability of the pressed paint product. In this test, a 10 g pan was dropped from 3 different heights: 5, 10 and 15 cm [29]. Each test was repeated ten times, and the results were recorded.

Stability test

The stability of the pressed paint product was evaluated under various conditions, including storage without sunlight exposure and exposure to sunlight combined with heating-cooling cycles (45 - 4 °C) for 6 cycles [30]. The samples were assessed visually at both 0 and 28 days, and color differences (ΔE) were quantified using a colorimeter based on CIE L^* , a^* , and b^* values immediately after preparation and after 28 days.

Toxicity test

The pressed paint product and sodium lauryl sulfate were dissolved and prepared for testing in cell culture medium. The sample was sterilized by filtering through a membrane with a pore size of 0.2 microns. The test sample solution was then diluted to the desired

concentration using sterilized cell culture medium. Subsequently, the cytotoxicity of the sample was evaluated using the sulforhodamine B (SRB) staining method. The results are expressed as the percentage of cell survival relative to the control group, with values presented as mean \pm standard deviation (S.D.) based on 4 independent experiments [31,32].

Statistical analysis

The statistical analysis for this experiment included calculating the percentage yield of extract and the maximum average absorbance values (λ Max) under both normal and mordant conditions. To compare means among sample groups, one-way ANOVA was performed, followed by Scheffé's post hoc test for multiple comparisons. Additionally, comparisons of λ Max between normal and mordant conditions were conducted using the Independent-Samples T-Test, and correlations were assessed using Pearson's correlation coefficient. All analyses were carried out using SPSS version 13, with statistical significance set at 0.05.

Results and discussion

Plant extraction

The study evaluated crude extract yields from 6 plant species (*Clitoria ternatea* L., *Scleropteryum pentandrum*, *Vitex pinnata* L., *Oroxylum indicum* L., *Xylia xylocarpa*, and *Careya sphaerica*) under different extraction conditions, including temperatures of 60 and 80 °C, extraction times of 30 - 90 min, and both standard and mordant-assisted methods. Extraction yields generally ranged from 9 to 13 % under standard conditions as shown in **Figure 1**. Notably, increasing the temperature to 80 °C and extending the extraction time to 90 min significantly improved yields for most species [33]. *Clitoria ternatea* L. achieved the highest yield (12.92 % ± 0.03), likely due to its distinctive cellular structure and high concentrations of water-soluble phytochemicals such as anthocyanins and phenolics. Higher temperatures increase cell wall permeability in this species, enhancing solvent penetration and extraction efficiency. These findings are consistent with previous studies, which report that *Clitoria ternatea* L. reliably outperforms other plants in extraction yield due to its unique cellular composition and favorable response to thermal processing [34,35], as summarized in **Tables 3** and **4**. Mordant-assisted extractions produced notably inferior results (3.16 - 4.57 %) regardless of species or parameters, while temperature significantly enhanced extraction efficiency. This improvement stems from increased cell wall permeability and reduces solvent viscosity at higher temperatures [36], confirming temperature's critical role in affecting compound solubility and enhancing mass transfer rates during extraction. However, excessive heat may degrade thermolabile compounds, necessitating careful temperature regulation to balance

efficiency and stability [37,38]. Plant species demonstrated significant increases in response variables ($p < 0.05$) at 60 °C, while all experimental conditions showed statistically significant increases ($p < 0.05$) at 80 °C. Extended extraction periods facilitate improved solute diffusion and optimize mass transfer between plant material and solvent, with research consistently showing positive correlations between extraction duration and yield, particularly when using polar solvents like ethanol or water. Nevertheless, while longer durations typically increase yields, they risk degrading bioactive compounds due to heat or enzymatic activity, highlighting the necessity of balancing extraction parameters to maximize efficiency while preserving compound integrity [39-41]. The extraction method critically influences both yield and quality, impacting industrial and research applications [42,43]. For *Clitoria ternatea* L., optimal extraction was achieved at 80 °C for 90 min under atmospheric pressure, yielding 12.92 %, consistent with previous studies. Comparatively, polyphenol extraction from rambutan peel at 60 °C for 34 min yielded 28.68 % [44]. The higher yield from *Clitoria ternatea* L. at 80 °C is attributed to improved solute solubility, enhanced mass transfer [45], and reduced solvent viscosity, which together promote cell wall disruption and greater release of intracellular compounds [46]. Increasing the extraction temperature from 60 to 80 °C for 90 min led to a 12.64 % increase in yield. The optimized conditions balance extraction efficiency and anthocyanin stability, reflecting patterns seen in other anthocyanin-rich species such as *Oryza sativa* L. Thus, extraction at 80 °C for 90 min maximizes yield while preserving compound integrity [47-49].

Table 3 The comparison of the percentage yield of extract under normal conditions.

Plant Species	Normal Parameter					
	% Yield (Mean) ± (S.D.)					
	Temp. 60 °C			Temp. 80 °C		
	30 min	60 min	90 min	30 min	60 min	90 min
<i>Clitoria ternatea</i> L.	11.38 ± 0.01 ^b	11.29 ± 0.01 ^c	11.47 ± 0.02 ^a	11.79 ± 0.02 ^b	11.37 ± 0.01 ^c	12.92 ± 0.03 ^a
<i>Scleropteryum pentandrum</i>	9.37 ± 0.04 ^c	9.42 ± 0.03 ^b	9.58 ± 0.05 ^a	9.26 ± 0.05 ^c	9.46 ± 0.09 ^b	9.76 ± 0.05 ^a
<i>Vitex pinnata</i> L.	9.37 ± 0.03 ^c	10.15 ± 0.04 ^a	9.79 ± 0.03 ^b	9.88 ± 0.06 ^b	9.16 ± 0.01 ^c	10.80 ± 0.04 ^a
<i>Oroxylum indicum</i> L.	10.81 ± 0.09 ^a	9.37 ± 0.03 ^c	10.54 ± 0.06 ^b	10.12 ± 0.07 ^c	10.25 ± 0.03 ^b	11.12 ± 0.06 ^a

Plant Species	Normal Parameter % Yield (Mean) ± (S.D.)					
	Temp. 60 °C			Temp. 80 °C		
	30 min	60 min	90 min	30 min	60 min	90 min
<i>Xylia xylocarpa</i>	10.81 ± 0.01 ^b	10.80 ± 0.02 ^b	11.13 ± 0.02 ^a	10.81 ± 0.02 ^b	10.48 ± 0.01 ^c	11.48 ± 0.02 ^a
<i>Careya sphaerica</i>	10.12 ± 0.01 ^a	9.72 ± 0.03 ^b	10.02 ± 0.01 ^a	9.40 ± 0.01 ^b	9.35 ± 0.01 ^c	10.14 ± 0.01 ^a

Note: Values in the same row with the same uppercase letter are not significantly different ($p > 0.05$).

Table 4 The comparison of the percentage yield of extract under conditions mordant (NaCl, 10 % w/w).

Plant Species	Mordant Parameter % Yield (Mean) ± S.D.					
	Temp. 60 °C			Temp. 80 °C		
	30 min	60 min	90 min	30 min	60 min	90 min
<i>Clitoria ternatea</i> L.	3.90 ± 0.01 ^b	3.95 ± 0.01 ^a	3.85 ± 0.01 ^c	4.18 ± 0.02 ^c	4.57 ± 0.01 ^a	4.36 ± 0.01 ^b
<i>Scleropyrum pentandrum</i>	3.29 ± 0.03 ^a	3.22 ± 0.04 ^b	3.16 ± 0.04 ^c	3.18 ± 0.03 ^a	3.19 ± 0.01 ^a	3.48 ± 0.03 ^a
<i>Vitex pinnata</i> L.	3.61 ± 0.01 ^a	3.47 ± 0.01 ^b	3.41 ± 0.01 ^c	3.72 ± 0.03 ^a	3.49 ± 0.01 ^b	3.73 ± 0.01 ^a
<i>Oroxylum indicum</i> L.	3.61 ± 0.01 ^b	4.14 ± 0.01 ^a	3.61 ± 0.02 ^b	3.98 ± 0.01 ^c	4.07 ± 0.02 ^b	4.30 ± 0.02 ^a
<i>Xylia xylocarpa</i>	3.91 ± 0.01 ^b	4.04 ± 0.01 ^a	3.79 ± 0.01 ^c	3.24 ± 0.01 ^c	3.85 ± 0.01 ^b	4.25 ± 0.01 ^a
<i>Careya sphaerica</i>	3.84 ± 0.02 ^a	3.48 ± 0.01 ^b	3.36 ± 0.01 ^c	3.59 ± 0.01 ^c	3.84 ± 0.01 ^b	4.04 ± 0.01 ^a

Note: Different letters (a, b and c) in the sam raw indicated significant differences ($p < 0.05$)

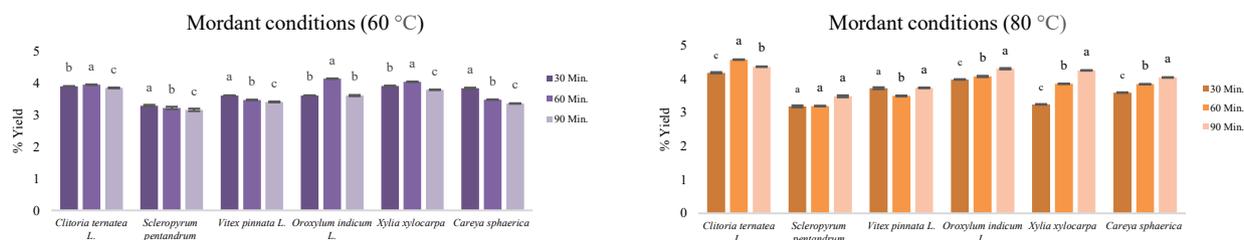


Figure 1 The comparison of the percentage yield of extract under mordant conditions at during times. Different letters (a, b and c) above the bars within the same plant species indicate significant differences ($p < 0.05$). Values with the same letter are not significantly different.

Absorption of plants using UV-Visible spectroscopy method

The study demonstrated that temperature, duration, and sodium chloride addition significantly influenced light absorption in plant pigment extracts using UV-Vis spectroscopy. Increasing temperature from 60 to 80 °C enhanced absorption due to improved pigment solubility and cell wall disruption. Extended extraction duration (9 min) increased absorption values, likely from prolonged solvent-pigment interaction.

Sodium chloride improved absorption by facilitating pigment release through ionic disruption of plant matrices, consistent across all species. Maximum absorption bands ranged between 80.444 - 20.490nm, aligning with chlorophyll and carotenoid absorbance profiles [50-52]. However, when examining specific extraction conditions, *Oroxylum indicum* L exhibited the highest absorption yield of 4.16 ± 0.09 at 60 °C for 60 min., suggesting this combination provides optimal extraction efficiency for this species. The optimum

condition yielding the highest absorption was observed for *Oroxylum indicum* L at 60 °C for 60 min (4.16 ± 0.09). This can be attributed to the specific thermostability profile of the bioactive compounds in this species. At 60 °C, cell wall disruption occurs efficiently, facilitating the release of intracellular compounds, while avoiding thermal degradation that becomes evident at higher temperatures (80 °C) or with prolonged extraction times (90 min). The plant contains significant amounts of polyphenols and flavonoids that exhibit peak extraction efficiency within this thermal range. Additionally, recent research suggests that medium- polarity compounds found in *Oroxylum indicum* L. require moderate heat treatment for optimal solubilization, as they balance between requiring sufficient thermal energy for extraction while maintaining structural integrity of heat- sensitive bioactive molecules [53]. This research examined the maximum average absorbance values of crude extracts from 6 plant species under normal conditions and with mordant addition (sodium chloride 10 % w/w). The results demonstrated that *Careya sphaerica* exhibited the highest absorbance values in all conditions, followed by *Clitoria ternatea* L. The optimal extraction condition for most plants was 60 °C for 60 - 90min. under normal

conditions, while all plants showed optimal results at 60 °C for 60min. when mordant was added. The addition of mordant significantly enhanced absorbance values across all plant extracts, with increases of 25 - 40 % compared to normal conditions. These findings align with recent research suggesting that mordant application improves dye fixation and color intensity while maintaining eco- friendly dyeing processes, making these plant extracts promising candidates for sustainable natural dyeing applications in the textile industry [54-60]. The absorption spectrum of plants, as shown in the provided figure, highlights the efficiency of chlorophyll pigments in capturing light for photosynthesis. Chlorophyll- a and chlorophyll- b exhibit peak absorption in the blue (400 - 500) and red (600 - 700 nm) regions, while green light (500 - 600nm) is less absorbed, leading to the characteristic green appearance of leaves [61,62]. Analysis of data comparing the absorbance of the mordant parameter and the absorbance of the normal parameter between temperatures of 60 and 80 °C at different times (30, 60 and 90 min) found that the group with significant differences ($p < 0.05$) is *Xylia xylocarpa* at 60 min (F- test = 95.61, p -value = 0.0103).

Table 5 The comparison of the maximum average absorbance values (λ Max) of extract under normal conditions.

Plant Species	λ Max	Absorbance of normal parameter (Mean \pm S.D.)					
		Temp. 60 °C			Temp. 80 °C		
		30 min	60 min	90 min	30 min	60 min	90 min
<i>Clitoria ternatea</i> L.	657.30	2.82 ± 0.01^c	3.18 ± 0.01^b	3.22 ± 0.01^a	2.90 ± 0.01^c	3.20 ± 0.01^b	3.22 ± 0.02^a
<i>Scleropyrum pentandrum</i>	403.50	0.57 ± 0.01^c	1.07 ± 0.01^b	1.28 ± 0.01^a	0.83 ± 0.01^c	1.18 ± 0.01^b	2.23 ± 0.01^a
<i>Vitex pinnata</i> L.	468.80	0.55 ± 0.01^c	0.90 ± 0.01^b	1.39 ± 0.01^a	0.60 ± 0.01^c	1.48 ± 0.01^b	3.09 ± 0.01^a
<i>Oroxylum indicum</i> L.	400.10	3.38 ± 0.03^c	4.16 ± 0.49^b	4.32 ± 0.58^a	2.38 ± 0.01^c	3.50 ± 0.06^b	3.75 ± 0.16^a
<i>Xylia xylocarpa</i>	357.10	0.97 ± 0.01^c	1.77 ± 0.01^b	2.54 ± 0.01^a	1.79 ± 0.01^c	2.26 ± 0.01^b	2.79 ± 0.01^a
<i>Careya sphaerica</i>	455.10	1.03 ± 0.01^c	1.07 ± 0.01^b	1.69 ± 0.01^a	1.99 ± 0.01^c	2.12 ± 0.01^b	2.71 ± 0.01^a

Note: The values are significantly different ($p < 0.05$).

Table 6 The comparison of the maximum average absorbance values (λ Max) of extract under mordant conditions (NaCl, 10 % w/w).

Plant Species	λ Max	Absorbance of mordant parameter					
		Mean \pm S.D.					
		Temp. 60 °C			Temp. 80 °C		
		30 min	60 min	90 min	30 min	60 min	90 min
<i>Clitoria ternatea</i> L.	658.80	3.80 \pm 0.01 ^c	4.22 \pm 0.06 ^b	4.52 \pm 0.63 ^a	3.98 \pm 0.01 ^c	4.48 \pm 0.01 ^b	4.91 \pm 0.04 ^a
<i>Scleropyrum pentandrum</i>	448.20	1.13 \pm 0.11 ^c	1.56 \pm 0.01 ^b	2.59 \pm 0.21 ^a	2.40 \pm 0.01 ^c	2.55 \pm 0.06 ^b	3.25 \pm 0.01 ^a
<i>Vitex pinnata</i> L.	471.60	1.37 \pm 0.01 ^c	2.09 \pm 0.01 ^b	2.71 \pm 0.01 ^a	1.92 \pm 0.06 ^c	2.32 \pm 0.23 ^b	2.88 \pm 0.06 ^a
<i>Oroxylum indicum</i> L.	450.20	3.87 \pm 0.03 ^c	4.64 \pm 0.04 ^b	4.70 \pm 0.19 ^a	3.69 \pm 0.07 ^c	3.93 \pm 0.57 ^b	4.20 \pm 0.01 ^a
<i>Xylia xylocarpa</i>	470.30	1.34 \pm 0.06 ^c	1.78 \pm 0.01 ^b	3.57 \pm 0.03 ^a	2.15 \pm 0.01 ^c	2.37 \pm 0.12 ^b	3.46 \pm 0.01 ^a
<i>Careya sphaerica</i>	490.10	1.97 \pm 0.01 ^c	2.51 \pm 0.01 ^b	3.41 \pm 0.01 ^a	2.45 \pm 0.01 ^c	3.27 \pm 0.01 ^b	3.36 \pm 0.01 ^a

Note: The values are significantly different ($p < 0.05$).

The results in **Tables 5 - 6** indicate that the absorbance values (λ Max) of plant extracts vary significantly under normal and mordant conditions, influenced by temperature, time, and the presence of NaCl (10 % w/w). Under normal conditions, *Oroxylum indicum* L. exhibited the highest absorbance at 80 °C after 90 min (3.75 \pm 0.16), while *Clitoria ternatea* L. also showed high stability across conditions. In mordant conditions, absorbance values increased overall, with *Oroxylum indicum* L. and *Clitoria ternatea* L. achieving the highest values at 80 °C after 90 min (4.20 \pm 0.01 and 4.91 \pm 0.04, respectively). The primary mechanism for enhancing dye adsorption and color fastness involves the formation of complex compounds between mordants and dye molecules. Metal ions in mordants interact with the dye's electron system, reducing overall energy and

altering light absorption properties [63]. Elevated temperatures improve dye adsorption by increasing dye exhaustion, with color strength peaking at 70 °C [64]. Extended durations (30, 60 and 90 min) further enhance adsorption, as shown in absorption spectra, due to improved penetration and more complete complex formation. This synergy of temperature and time ensures greater dye stability and durability, as prolonged exposure allows deeper molecular interactions and robust complexation between dyes and mordants. The comparison of maximum absorption wavelengths (λ Max) between normal and mordant conditions shows that the mordant condition has a higher absorbance value because it is darker in color in all samples, as shown in **Figure 2**.

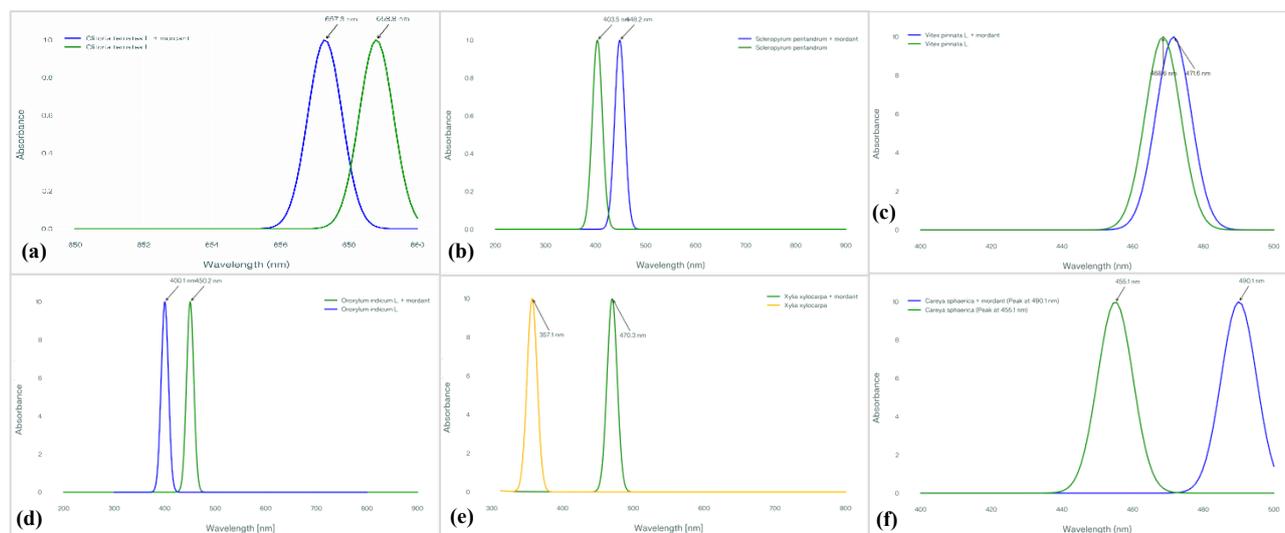


Figure 2 Comparison of λ_{Max} absorption spectra under normal and mordant conditions. The absorbance spectra for each extract are as follows: (a) *Clitoria ternatea* L., (b) *Scleropyrum pentandrum*, (c) *Vitex pinnata* L., (d) *Oroxylum indicum* L., (e) *Xylia xylocarpa*, and (f) *Careya sphaerica*.

The statistical analysis comparing λ_{Max} values between normal and mordant conditions for 6 plant species revealed no significant differences, as indicated by p -values greater than 0.05 for all species. Pearson correlation coefficients varied widely, ranging from strong negative to strong positive values, but none reached statistical significance. This suggests that the use of mordant does not significantly alter the λ_{Max} of the plant extracts studied, and the relationship between normal and mordant conditions is inconsistent across different species. Overall, the findings indicate that mordant treatment does not have a significant impact on the λ_{Max} parameter in these plant species, as shown in **Table 7**.

This study used UV-Vis spectroscopy to analyze light absorption in plant-derived dyes under different temperatures, extraction times, and sodium chloride (NaCl) concentrations. The results showed that increasing the temperature to 80 °C and extending the extraction time to 90 min boosted light absorption by 50 - 1.5 times. Adding 10 % w/v NaCl increased absorbance values by 25 - 40 %, along with improved color intensity and dye stability. *Oryza sativa* L. showed a peak absorbance of 4.91 ± 0.04 under optimal conditions, while *Careya sphaerica* reached 3.36 ± 0.01 , indicating efficient extraction of chlorophyll and

carotenoid pigments. The maximum absorbance between 400 - 490 nm corresponded to anthocyanin and carotenoid spectra, responsible for red-orange-yellow hues. NaCl functioned as an effective mordant by promoting ionic interactions [65], which strengthened dye-fiber binding and colorfastness.

These findings highlight the importance of optimizing extraction parameters to achieve high dye performance and pigment stability. Sodium chloride facilitated extraction through 2 main mechanisms. First, chloride ions (Cl^-) disrupted cell wall structures, releasing pigments trapped in vacuoles and chloroplasts [65,66]. Second, sodium ions (Na^+) formed bonds with negatively charged pigments, improving stability and preventing aggregation during extraction. This approach ensured efficient pigment recovery while maintaining the structural integrity of the dye.

Comparison with previous studies confirmed that NaCl can help maintain color stability under acidic conditions. It was also found that increasing the temperature above 80 °C or extending the extraction time beyond 90 min could lead to pigment degradation, as indicated by the high standard deviation (S.D.) found in *Oroxylum indicum* L. (4.32 ± 0.58). This instability may be due to the presence of heat-sensitive compounds in these plant species

Table 7 The analysis of statistical comparison between λ Max normal and mordant parameter.

Plant Species	λ Max		Statistical comparison between λ Max mordant and normal parameter	
	Normal	Mordant	Pearson Correlation	p-value
<i>Clitoria ternatea</i> L.	657.30	658.80	-0.327	0.788
<i>Scleropyrum pentandrum</i>	403.50	448.20	-0.961	0.179
<i>Vitex pinnata</i> L.	468.80	471.60	0.866	0.333
<i>Oroxylum indicum</i> L.	400.10	450.20	-0.500	0.667
<i>Xylia xylocarpa</i>	357.10	470.30	0.327	0.788
<i>Careya sphaerica</i>	455.10	490.10	s0.052	0.967

Note: The values are not significantly different ($p > 0.05$).

Color measurement

The comparative $L^*a^*b^*$ values of 6 plant species at 60 and 80 °C under normal conditions. In both tables, the lightness (L^*) value generally decreases with longer heating durations, indicating darkening over time. The values (red-green axis) show minor fluctuations, while the b values (yellow-blue axis) tend to increase for most species, suggesting a shift toward warmer tones. Comparing the 2 tables, higher temperatures (80 °C) accelerate these changes, as evidenced by lower L^* values and more pronounced shifts in a and b values at equivalent time intervals. The results suggested temperature stress rapidly initiates a cascade of molecular and metabolic responses in plants. These responses include the breakdown of pigments and the activation of various stress-related pathways [67]. However, with extended or severe heat exposure, overall pigmentation typically declines, resulting in lighter or modified coloration. The complex interplay between pigment synthesis, degradation processes, and cellular damage fundamentally explains the observed changes in color metrics ($L^*a^*b^*$) that correlate with increasing temperature intensity and exposure duration. Heating duration and temperature demonstrate significant influence on the visual properties of plant materials. **Tables 8 and 9** present Lab* values of various plant species under normal conditions at 60 and 80 °C, while **Tables 10 and 11** display the corresponding values under mordant conditions at the same temperatures. At both temperature levels, substantial variations in lightness (L^*), redness/greenness (a^*), and yellowness/blueness (b^*) parameters are evident across

different plant species and treatment durations (30, 60 and 90 min). *Clitoria ternatea* L., for instance, exhibits progressive decrease in lightness (L^*) values concurrent with increased blueness (negative b^*) as mordanting time extends at both temperature settings. Conversely, *Oroxylum indicum* and *Xylia xylocarpa* display elevated L^* , a^* , and b^* values, indicating brighter and warmer color tones. Comparative analysis between the 2 conditions reveals that higher temperatures (80 °C) generally reduce L^* values while intensifying a^* and b^* parameters for most plant species, suggesting enhanced dye fixation or deeper color absorption. The correlation between temperature, duration, and color intensity indicates that elevated temperatures combined with extended mordanting periods tend to produce more vivid and saturated colorations. These findings underscore the critical importance of optimizing temperature and duration parameters to achieve desired dyeing outcomes for specific plant species. Statistical comparison of L^* , a^* , and b^* values further elucidate the differential effects between normal and mordant conditions. Statistical analysis revealed significant color parameter differences ($p < 0.05$) among several plant species, particularly *Clitoria ternatea* L., *Scleropyrum pentandrum*, and *Vitex pinnata* L., when subjected to mordanting at 80 °C. This testing identified optimal dyeing conditions for maximum color darkness across six plant species, with mordant application at 80 °C for 90 min proving most effective for five species: *Clitoria ternatea* L. (L^* 38.14), *Vitex pinnata* L. (L^* 57.02), *Oroxylum indicum* L. (L^* 70.02), *Xylia xylocarpa* (L^* 56.58), and *Careya sphaerica* (L^* 52.75), yielding

minimal L* values and elevated a* and b* values for enhanced color intensity. Notably, *Sclerocarya pentandra* exhibited unique temperature sensitivity, achieving optimal darkness at 60 °C with mordant (L* 62.59). These findings demonstrate species-specific optimization requirements while confirming the general

effectiveness of higher temperatures (80 °C) and mordant application for enhancing color depth in plant-based dyes, as evidenced in the experimental data presented in **Tables 12** and **13**.

Table 8 The comparison of L*a*b* color values of plant species under normal conditions at 60 °C.

Plant Species	Normal Parameter (Mean ± S.D.)								
	Temp. 60 °C								
	30 min			60 min			90 min		
	L*	a*	b*	L*	a*	b*	L*	a*	b*
<i>Clitoria ternatea</i> L.	59.34 ± 0.13	-1.75 ± 0.04	-5.07 ± 0.13	57.13 ± 0.19	-1.64 ± 0.05	-5.41 ± 0.17	56.31 ± 0.66	-1.55 ± 0.08	-5.85 ± 0.18
<i>Scleropyrum pentandrum</i>	80.44 ± 0.04	2.79 ± 0.05	10.64 ± 0.10	79.81 ± 0.02	2.70 ± 0.05	11.13 ± 0.12	78.82 ± 0.06	2.62 ± 0.05	12.17 ± 0.16
<i>Vitex pinnata</i> L.	77.21 ± 0.01	1.26 ± 0.04	25.49 ± 0.14	76.12 ± 0.04	1.73 ± 0.05	27.40 ± 0.25	74.21 ± 0.10	2.49 ± 0.51	28.10 ± 0.48
<i>Oroxylum indicum</i> L.	82.58 ± 0.02	1.27 ± 0.04	18.52 ± 0.12	81.99 ± 0.14	1.29 ± 0.03	18.66 ± 0.04	79.33 ± 0.06	1.59 ± 0.09	18.74 ± 0.23
<i>Xylia xylocarpa</i>	84.85 ± 0.12	5.79 ± 0.08	20.11 ± 0.23	78.29 ± 0.27	10.24 ± 0.19	22.86 ± 0.19	63.30 ± 0.02	19.22 ± 0.04	24.97 ± 0.09
<i>Careya sphaerica</i>	64.71 ± 0.23	12.78 ± 0.54	19.63 ± 0.68	63.21 ± 0.19	19.46 ± 0.18	23.55 ± 0.41	62.09 ± 0.29	22.62 ± 0.25	25.81 ± 0.66

Table 9 The comparison of L*a*b* color values of plant species under normal conditions at 80 °C.

Plant Species	Normal Parameter (Mean ± S.D.)								
	Temp. 80 °C								
	30 min			60 min			90 min		
	L*	a*	b*	L*	a*	b*	L*	a*	b*
<i>Clitoria ternatea</i> L.	55.74 ± 0.08	-1.54 ± 0.13	-6.10 ± 0.09	52.20 ± 0.15	-1.43 ± 0.11	-6.69 ± 0.23	48.01 ± 0.08	-1.40 ± 0.05	-6.79 ± 0.05
<i>Scleropyrum pentandrum</i>	74.46 ± 0.09	2.40 ± 0.03	12.98 ± 0.11	74.22 ± 0.04	1.99 ± 0.04	13.05 ± 0.06	73.69 ± 0.01	1.95 ± 0.02	14.79 ± 0.13

Plant Species	Normal Parameter (Mean ± S.D.)								
	Temp. 80 °C								
	30 min			60 min			90 min		
	L*	a*	b*	L*	a*	b*	L*	a*	b*
<i>Vitex pinnata</i> L.	74.35 ± 0.01	2.80 ± 0.03	29.48 ± 0.05	73.77 ± 0.04	2.90 ± 0.02	31.03 ± 0.11	72.52 ± 0.03	2.99 ± 0.01	35.54 ± 0.20
<i>Oroxylum indicum</i> L.	80.24 ± 0.02	1.68 ± 0.05	18.82 ± 0.07	77.95 ± 0.02	1.99 ± 0.02	18.92 ± 0.15	75.61 ± 0.95	2.01 ± 0.02	19.02 ± 0.12
<i>Xylia xylocarpa</i>	82.55 ± 0.01	9.84 ± 0.04	20.34 ± 0.06	75.72 ± 0.06	12.46 ± 0.02	22.44 ± 0.08	60.25 ± 0.07	18.25 ± 0.04	24.27 ± 0.27
<i>Careya sphaerica</i>	60.13 ± 0.23	14.75 ± 0.48	20.28 ± 0.03	59.84 ± 0.99	22.03 ± 0.47	30.69 ± 0.97	55.94 ± 0.17	24.75 ± 0.12	37.65 ± 0.11

The development of pressed paint products

The experiment successfully demonstrated the potential of natural pigments extracted from 6 plant species - *Clitoria ternatea* L., *Carexa sphaerica*, *Xylia xylocarpa*, *Sclerocarya pentandra*, *Vitex pinnata* L., and *Oroxylum indicum* L.- for use in pressed paint products. The optimal conditions for extraction involved temperatures of 60 - 80 °C and durations of 90 min, with the application of mordants enhancing color intensity and stability. The resulting colors ranged from deep blue (*Clitoria ternatea* L.) to earth tones such as medium brown (*Sclerocarya pentandra* and *Xylia xylocarpa*), yellow (*Vitex pinnata* L.), light brown (*Oroxylum indicum* L.), and dark brown (*Carexa sphaerica*). The

pressed paint products exhibited fine texture, smooth application, ease of blending, and clear pigmentation, as shown in **Figure 3**.

Result of stability test

Compression test of the formulation

The compression test results demonstrated that all formulations of the colored powders exhibited excellent adhesion to the pan surface. After subjecting the powders to approximately 30 repetitions of the drop test on a table surface, no cracking or dispersion of the powders was observed. This indicates that the formulations possess robust mechanical integrity and are suitable for applications requiring high durability.

Table 11 The comparison of L*a*b* color values of plant species under mordant conditions at 80 °C.

Plant Species	Mordant Parameter (Mean ± S.D.)								
	Temp. 80 °C								
	30 min			60 min			90 min		
	L*	a*	b*	L*	a*	b*	L*	a*	b*
<i>Clitoria ternatea</i> L.	40.36 ± 0.13	-1.03 ± 0.02	-7.99 ± 0.05	40.25 ± 0.06	-1.02 ± 0.12	-8.82 ± 0.14	38.14 ± 0.12	-1.00 ± 0.15	-8.90 ± 0.23
<i>Scleropyrum pentandrum</i>	61.43 ± 0.01	1.03 ± 0.05	16.60 ± 0.08	60.21 ± 0.02	1.01 ± 0.06	16.94 ± 0.06	55.30 ± 0.06	1.00 ± 0.05	17.06 ± 0.13
<i>Vitex pinnata</i> L.	67.24 ± 0.03	3.80 ± 0.03	44.23 ± 0.05	60.14 ± 0.04	3.95 ± 0.03	49.07 ± 0.09	57.02 ± 0.07	3.99 ± 0.07	50.19 ± 0.32
<i>Oroxylum indicum</i> L.	72.15 ± 0.01	2.39 ± 0.90	20.36 ± 0.70	70.92 ± 0.02	2.41 ± 0.02	21.37 ± 0.02	70.02 ± 0.02	2.52 ± 0.03	22.45 ± 0.29
<i>Xylia xylocarpa</i>	73.64 ± 0.05	12.05 ± 0.04	24.71 ± 0.17	59.02 ± 0.09	22.49 ± 0.05	32.81 ± 0.39	56.58 ± 0.08	22.53 ± 0.03	45.47 ± 0.05
<i>Careya sphaerica</i>	58.24 ± 0.24	24.15 ± 0.11	5.02 ± 0.12	56.78 ± 0.96	26.01 ± 0.11	13.51 ± 0.37	52.75 ± 0.73	29.16 ± 0.35	22.10 ± 0.49

Table 12 The comparison of statistical analysis between L*a*b* each condition.

Parameter	Plant Species	Normal (60 and 80 °C)			Mordant (60 and 80 °C)			Normal and Mordant (60 and 60 °C)			Normal and Mordant (80 and 80 °C)		
		L*	a*	b*	L*	a*	b*	L*	a*	b*	L*	a*	b*
F-test	<i>Clitoria ternatea</i> L.	0.071	0.237	0.006	3.831	7.663	9.924	0.028	0.042	0.051	27.89	102.49	31.88
	<i>Scleropyrum pentandrum</i>	0.106	0.049	0.002	6.001	9.163	6.267	0.006	0.016	0.021	64.39	58.30	28.77
	<i>Vitex pinnata</i> L.	0.245	0.000	0.013	10.185	5.533	16.782	0.008	0.012	0.014	15.47	162.63	37.61
	<i>Oroxylum indicum</i> L.	0.274	0.014	0.093	3.917	13.517	5.590	0.006	0.007	0.002	21.99	22.91	16.65
	<i>Xylia xylocarpa</i>	0.286	0.009	0.242	0.053	0.312	0.678	0.0005	0.001	0.010	1.33	1.65	3.80
	<i>Careya sphaerica</i>	0.351	0.004	0.372	0.890	1.766	3.642	0.00009	0.013	0.044	1.63	3.18	5.14
p-value	<i>Clitoria ternatea</i> L.	0.803	0.652	0.944	0.122	0.050	0.035*	0.875	0.847	0.832	0.0062*	0.0005*	0.0048*
	<i>Scleropyrum pentandrum</i>	0.761	0.836	0.969	0.070	0.039*	0.067	0.944	0.906	0.891	0.0013*	0.0016*	0.0058*
	<i>Vitex pinnata</i> L.	0.647	0.991	0.913	0.033*	0.078	0.015*	0.934	0.917	0.916	0.0171*	0.0002*	0.0036*
	<i>Oroxylum indicum</i> L.	0.628	0.911	0.776	0.119	0.021*	0.077	0.944	0.936	0.963	0.0094*	0.0087*	0.0151*
	<i>Xylia xylocarpa</i>	0.621	0.928	0.649	0.829	0.606	0.456	0.983	0.974	0.927	0.3138	0.2678	0.1231
	<i>Careya sphaerica</i>	0.586	0.954	0.575	0.399	0.255	0.129	0.993	0.913	0.844	0.2709	0.1490	0.0860

Note: *Significant differences ($p < 0.05$).

Table 13 The optimal condition in this experiment.

Plant Species	Optimal Condition	Temp(°C) & Time (min)	L* Value	Color Intensity
<i>Clitoria ternatea</i> L.	Mordant	80, 90	38.14	Dark blue
<i>Sclerocarya pentandra</i>	Mordant	60, 90	62.59	Medium brown
<i>Vitex pinnata</i> L.	Mordant	80, 90	57.02	Yellow
<i>Oroxylum indicum</i> L.	Mordant	80, 90	70.02	Light brown
<i>Xylia xylocarpa</i>	Mordant	80, 90	56.58	Medium brown
<i>Careya sphaerica</i>	Mordant	80, 90	52.75	Dark brown

**Figure 3** Pressed paint products: (a) *Clitoria ternatea* L., (b) *Careya sphaerica*, (c) *Xylia xylocarpa*, (d) *Scleropyrum pentandrum*, (e) *Vitex pinnata* L., and (f) *Oroxylum indicum* L.

Stability test of various conditions

The stability test results demonstrated consistent physical stability across all tested conditions during the 28-day observation period. Visual inspections under 4 conditions (no sunlight, sunlight exposure, 45 °C oven, and 4 °C) showed no observable changes in color or appearance at 14- day and 28- day intervals. The quantitative analysis revealed that ΔE values were < 1 in all conditions except the 45 °C oven; 45 °C oven samples showed $\Delta E = 2 - 3.5$ (indicating measurable color shift); increased L^* values suggested brightness changes as the primary factor. Notably, the color difference in oven-treated samples ($\Delta E 2 - 3.5$) remained below the visual detection threshold for human observers. This discrepancy between instrumental measurements and visual assessment highlights the importance of combining both quantitative and

qualitative methods in stability testing. The results suggest the formulation maintains adequate stability under typical storage conditions, though thermal stress at 45 °C may induce subtle physicochemical changes detectable only through instrumentation.

Drop test

The drop test results indicate that the packaging effectively protects the product under rigorous conditions. After being dropped 10 times in specified directions from a height corresponding to the weight of the packaged product, all packages remained intact without any visible damage, such as dents, scratches, cracks, breaks, or loose parts. This demonstrates the durability and reliability of the packaging design in maintaining product safety during handling and transportation, as illustrated in **Figure 4**.

The application of press colors in creative painting

The creative painting as shown in **Figure 5** demonstrates the successful application of natural dyes extracted from 6 types of plants, combined with mordant sodium chloride, to create vivid and imaginative

artwork. This approach highlights the potential of using eco-friendly, plant-based colors in artistic practices, achieving aesthetically pleasing results while promoting sustainable and natural alternatives to synthetic pigments.



Figure 4 The pressed paint products in packaging.



Figure 5 Creative painting using natural colors extracted from 6 plant species.

Table 14 The percent of cell viability of human skin fibroblast cells.

Sample	%Cell Viability				
	Conc. (mg/mL)				
	0.001	0.01	0.1	1	10
<i>Clitoria ternatea</i> L.	97.85 ± 1.54	93.81 ± 3.75	93.49 ± 3.24	87.31 ± 3.16	81.93 ± 1.68
<i>Scleropyrum pentandrum</i>	94.91 ± 3.24	94.02 ± 2.47	92.38 ± 1.29	91.30 ± 3.97	78.43 ± 2.56
<i>Vitex pinnata</i> L.	96.49 ± 2.39	94.75 ± 0.52	91.87 ± 1.16	90.89 ± 1.27	80.69 ± 1.30
<i>Oroxylum indicum</i> L.	95.05 ± 1.32	93.93 ± 1.46	92.37 ± 1.57	82.74 ± 1.28	77.34 ± 1.27
<i>Xylia xylocarpa</i>	94.94 ± 1.47	93.46 ± 1.95	91.93 ± 1.35	85.94 ± 1.43	79.32 ± 1.60
<i>Careya sphaerica</i>	96.62 ± 1.89	94.36 ± 1.92	93.35 ± 1.27	89.75 ± 1.75	82.53 ± 1.44
<i>Clitoria ternatea</i> L.	97.85 ± 1.54	95.64 ± 1.24	92.35 ± 1.49	85.48 ± 1.95	84.83 ± 1.38
Sodium lauryl sulfate	93.07 ± 1.33	90.47 ± 2.84	4.22 ± 0.49	2.62 ± 0.19	1.86 ± 0.06

Note: The values are shown as mean ± S.D., based on 4 repeated experiments.

Toxicity test

The cell viability data demonstrates varying cytotoxic effects of plant extracts and compounds on human skin fibroblast cells across different concentrations. At lower concentrations (0.001 - 1.0 mg/mL), most samples exhibited minimal toxicity with cell viability above 90 %, indicating good biocompatibility. Notably, *Oroxylum indicum* L. showed the highest cell viability (65.95± 32.1 %) at 0.001mg/mL, suggesting its potential safety for topical applications. However, as concentrations increased to -1 10mg/mL, all samples demonstrated dose-dependent cytotoxicity, with sodium lauryl sulfate showing the most severe effect (only 86.1± 06.0 % viability at 10 mg/mL), serving as a positive control for cytotoxicity as shown in **Figure 6**. Among the plant extracts, *Vitex pinnata* maintained relatively higher cell viability (69.80± 30.1 %) even at 10mg/mL, as shown in **Table 14**. Recent research relates to sodium chloride as an eco-

friendly mordant alternative in natural dyeing processes, addressing growing concerns about the toxicity of conventional mordants identified sodium chloride as a non-toxic fixator that provides good colorfastness while minimizing environmental impact compared to metal-based mordants [68,69]. Furthermore, demonstrated that natural mordants like sodium chloride can effectively replace toxic metal salts in textile dyeing applications, showing promising results particularly with cotton and jute yarns, though with some trade-offs in wash fastness properties More recently, A research confirmed that sodium chloride serves as an effective mordant for natural dye extraction, promoting sustainable textile production without the need for hazardous chemicals typically used in conventional dyeing processes. These findings collectively support the shift toward chemical-free dyeing methodologies that maintain color efficacy while reducing toxicity concerns [70].

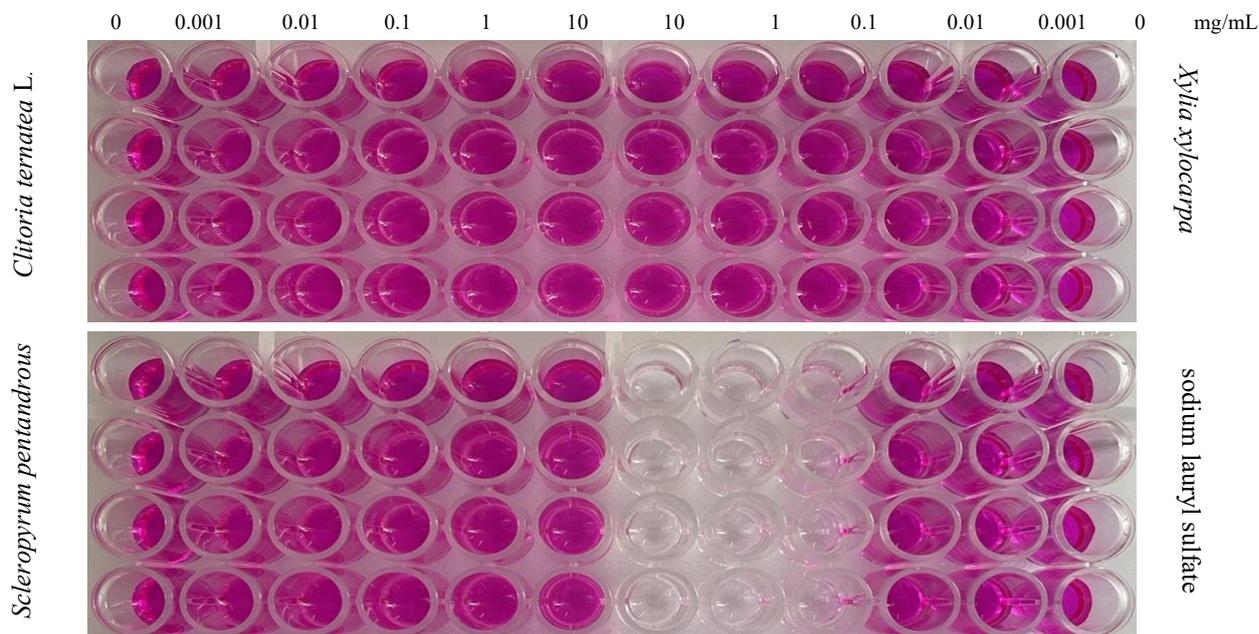


Figure 6 The example of toxicity test of extracts and sodium lauryl sulfate on human dermal fibroblast cells using the sulforhodamine B staining method.

Conclusions

The optimal extraction conditions in this study were determined to be 60 °C for 60 min. when using sodium chloride as a mordant, with this combination

providing enhanced pigment extraction efficiency across all 6 plant species tested. The addition of sodium chloride (10 % w/w) significantly improved absorbance values by 25 - 40 % compared to standard conditions,

resulting in more vibrant colorations with clear pigmentation. Notably, while standard extraction conditions yielded higher crude extract percentages (9.16 - 12.92 %), the mordant-assisted approach proved superior for color intensity and stability. The natural pigment extracts demonstrated excellent physical properties when formulated into pressed paint products, exhibiting fine texture, smooth application, and outstanding adhesion properties. Stability testing confirmed product durability under various environmental conditions, with minimal color changes (ΔE values < 1) in most scenarios over a 28-day period. This research validates sodium chloride as an effective, eco-friendly mordant alternative for natural dye extraction that result in this approach yields bright, durable pigments with reduced toxicity, suitable for cosmetics and art products. Future work should focus on broader commercial applications and long-term safety to promote sustainable industry practices.

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Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that Quillbot, an AI-based language editing tool, was used to improve the English language and enhance the readability of this manuscript.

CRedit Author Statement

Nongnuch Klinpikul: Responsible for the conceptualization of the study and the development of the methodology. **Wongnapa Nakyai:** Managed data curation and took the lead in preparing the original draft of the manuscript. **Somchye Dittapohn:** Contributed to the visualization of data and conducted the investigation. **Wannisa Keawbankrud:** Provided supervision and was actively involved in writing, reviewing, and editing the manuscript.

References

- [1] E Di Salvo, G Lo Vecchio, R De Pasquale, L De Maria and R Tardugno. Natural pigments production and their application in food, health and other industries. *Nutrients* 2023; **15(8)**, 1923.
- [2] L Chungkrang and S Bhuyan. Natural dye sources and its applications in textiles: A brief review. *International Journal of Current Microbiology and Applied Sciences* 2020; **9(10)**, 261-269.
- [3] N Li, Q Wang, J Zhou, S Li, J Liu and H Chen. Insight into the progress on natural dyes: Sources, structural features, health effects, challenges, and potential. *Molecules* 2022; **27(10)**, 3291.
- [4] D Hamd and A Hassabo. Various natural dyes from different sources. *Journal of Textile Coloration and Polymer Science* 2021; **18(2)**, 171-190.
- [5] MR Repon, B Dev, MA Rahman, S Jurkonienė, A Haji, MA Alim and E Kumpikaitė. Textile dyeing using natural mordants and dyes: A review. *Environmental Chemistry Letters* 2024; **22(3)**, 1473-1520.
- [6] FS Abd El-Samie, NF Ali, IS Abd El-Salam and Y Bayad. Extraction of eco-friendly natural dye from Clitoria plant and its application in textile by economic methods. *Discovery* 2022; **58(323)**, 1204-1212.
- [7] TK Ajith Babu, KK Athulya and KS Sandeep. Formulation and evaluation of a herbal topical anti-inflammatory gel containing methanolic leaf extract of *Scleropyrum Pentandrum* (Dennst.) Mabb. *International Journal of Pharmaceutical Research and Applications* 2022; **7(6)**, 1709-1719.
- [8] A Sanai, T Nachai and K Merat. Extraction of natural dye from *Oroxylum Indicum* (L.) Kurz by using different solvents for Thai eri silk dyeing. *Journal of Applied Research in Science and Technology* 2023; **22(2)**, 251099.
- [9] MG Rasul. Conventional extraction methods use in medicinal plants, their advantages and disadvantages. *International Journal of Basic Sciences and Applied Computing* 2018; **2(6)**, 10-14.
- [10] D Rini, I Ratnawati, A Wisesa and L Sidiyawati. Mahogany wood as natural dyes for textile colouring: Experiment at home industry Batik Wonorejo Village Singosari Malang Regency. *Proceedings of the International Seminar on Language, Education, and Culture* 2023; **742**, 66-74.

- [11] M Jayakumari, M Prakash, SR Kulkarni, J Hayavadana, K Srinivasulu, T Rajpurohit and M Vijayasathy. Study of effect of mordants on cotton fabric using dye developed from Antigonon leptopus. *African Journal of Biomedical Research* 2024; **27(9)**, 3164-3167.
- [12] QH Ramli, R Mohd Hassan and N Mohd Nor. Dyeing of textile using different mordants, mordanting techniques and their effects on fastness properties. *ASEAN Journal of Life Sciences* 2021; **1(2)**, 64-67.
- [13] A Onal, KC Tumbul and S Nached. Investigation of dyeing properties of different fabric species with *Curcuma longa* extracts. *Revue Roumaine de Chimie* 2020; **65(11)**, 983-988.
- [14] N Vuthiganond, P Chitichotpanya, M Nakpathom and R Mongkholrattanasit. Ecological dyeing of acrylic yarn with colorant derived from natural lac dye. *Journal of Metals, Materials and Minerals* 2022; **32(4)**, 71-78.
- [15] N Pujeeb. The study of differences in color adhesion of plants: Natural mordant experiments with the eco-printing technique. *Silpa Bhirasri Journal of Fine Arts* 2023; **11(1)**, 1-18.
- [16] H Benli. Bio-mordants: A review. *Environmental Science and Pollution Research International* 2024; **31(14)**, 20714-20771.
- [17] R Chakraborty, AK Samanta, PS Vankar and P Samanta. Review on extraction, mordanting and application of natural dyes on jute. *Current Trends in Fashion Technology and Textile Engineering* 2022; **7(4)**, 555719.
- [18] MN Indira, J Sandra, AS Aleena and KK Anuja. Impact of mordants on fabrics dyed with natural dyes: A case study. *African Journal of Biological Sciences* 2024; **6(14)**, 4106-4115.
- [19] IS Che Sulaiman, M Basri, HR Fard Masoum, WJ Chee and SE Ashari. Effects of temperature, time, and solvent ratio on the extraction of phenolic compounds and the anti-radical activity of *Clinacanthus nutans* Lindau leaves by response surface methodology. *Chemistry Central Journal* 2017; **11(1)**, 54.
- [20] N Amirova and MZ Abdulkarimova. Possibility of intensive dyeing of cotton fabric with natural dyes. *International Journal of Research* 2017; **4(2)**, 158-160.
- [21] H Benli. Bio-mordants: A review. *Environmental Science and Pollution Research* 2024; **31(14)**, 20714-20771.
- [22] P Theamdee and T Wongsard. Development of screen inks from black soil and red soil using natural mordant. *RMUTI Journal Humanities and Social Sciences* 2024; **17(1)**, 27-37.
- [23] A Krakowska-Sieprawska, A Kielbasa, K Rafińska, M Ligor and B Buszewski. Modern methods of pre-treatment of plant material for the extraction of bioactive compounds. *Molecules* 2022; **27(3)**, 730.
- [24] AR Abubakar and M Haque. Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. *Journal of Pharmacy and Bioallied Sciences* 2020; **12(1)**, 1-10.
- [25] JP Maran, V Sivakumar, K Thirugnanasambandham and R Sridhar. Extraction of natural anthocyanin and colors from pulp of jamun fruit. *Journal of Food Science and Technology* 2015; **52(6)**, 3617-3626.
- [26] S RoofigariHaghighat, A Shirinfekr, R AzadiGonbad and A Seraji. Investigation on natural color extraction from black tea waste. *Journal of Medicinal Plants and By-Products* 2020; **1(1)**, 1-6.
- [27] SM Bae, MG Cho, GT Hong and JY Jeong. Effect of NaCl concentration and cooking temperature on the color and pigment characteristics of presalted ground chicken breasts. *Korean Journal of Food Science and Animal Resources* 2018; **38(2)**, 417-430.
- [28] ABD Nandiyanto, R Ragadhita and M Aziz. How to calculate and measure solution concentration using UV-Vis spectrum analysis: Supporting measurement in the chemical decomposition, photocatalysis, phytoremediation, and adsorption process. *Indonesian Journal of Science and Technology* 2023; **8(2)**, 345-362.
- [29] NZA Baharuddin and NT Kofli. Study on compact powder formulation from fermented rice powder (bedak sejuk). *Journal of Biochemistry, Microbiology and Biotechnology* 2022; **10(1)**, 9-12.
- [30] A Azeem, M Rizwan, FJ Ahmad, Z Iqbal, RK Khar, M Aqil and S Talegaonkar. Nanoemulsion

- components screening and selection: A technical note. *Journal of the American Association of Pharmaceutical Scientists* 2009; **10(1)**, 69-76.
- [31] V Vichai and K Kirtikara. Sulforhodamine B colorimetric assay for cytotoxicity screening. *Nature Protocols* 2006; **1(3)**, 1112-1116.
- [32] International Organization for Standardization. *Biological evaluation of medical devices - Part 5: Tests for in vitro cytotoxicity*. International Organization for Standardization, Geneva, Switzerland, 2009.
- [33] Kusumiyati, N Yuniati, YE Maulana and A Karuniawan. Yield and quality extract of *Clitoria ternatea* accessions with different drying methods. *International Journal on Advanced Science, Engineering and Information Technology* 2022; **12(5)**, 1805-1811.
- [34] NN Hasanah, E Mohamad Azman, A Rozzamri, NH Zainal Abedin and MR Ismail-Fitry. A systematic review of butterfly pea flower (*Clitoria ternatea* L.): Extraction and application as a food freshness pH-indicator for polymer-based intelligent packaging. *Polymers* 2023; **15**, 2541.
- [35] EJ Jeyaraj, YY Lim and WS Choo. Extraction methods of butterfly pea (*Clitoria ternatea*) flower and biological activities of its phytochemicals. *Journal of Food Science and Technology* 2021; **58(6)**, 2054-2067.
- [36] B Klaykruayat, N Vuthiganond and P Chitichotpanya. Optimization of ultrasound-assisted anthocyanin extraction from black rice bran for simultaneous coloring, UV protection, and antioxidant silk finishes. *Journal of Current Science and Technology* 2025; **15(1)**, 86.
- [37] YA Bhadange, J Carpenter and VK Saharan. A comprehensive review on advanced extraction techniques for retrieving bioactive components from natural sources. *American Chemical Society Omega* 2024; **9(29)**, 3127-3129.
- [38] D Kalompatsios, AI Ionescu, V Athanasiadis, T Chatzimitakos, M Mantiniotou and K Kotsou. Maximizing bioactive compound extraction from mandarin (*Citrus reticulata*) peels through green pretreatment techniques. *Oxygen* 2024; **4(3)**, 307-324.
- [39] M Chaowalit and L Chitradee. Effect of temperature and duration time of maceration on nitrate content of *Vernonia cinerea* (L.) Less.: Circumscribed central composite design and method validation. *International Journal of Food Science* 2019; **2019(1)**, 1281635.
- [40] MH Haido, AH Matti and SM Taher. Optimization of extraction conditions of bioactive compounds from Kurdistan species *Urtica dioica*. *Cureus* 2024; **16(5)**, e61146.
- [41] J Hepperle, D Dörk, A Barth, B Taşdelen and M Anastassiades. Studies to improve the extraction yields of incurred pesticide residues from crops using the QuEChERS method. *Journal of Association of Official Agricultural Chemists International* 2015; **98(2)**, 450-463.
- [42] S Cao, J Liang, M Chen, C Xu, X Wang, L Qiu, X Zhao and W Hu. Comparative analysis of extraction technologies for plant extracts and absolutes. *Frontiers in Chemistry* 2025; **13**, 1536590.
- [43] A Borges, H José, V Homem and M Simões. Comparison of techniques and solvents on the antimicrobial and antioxidant potential of extracts from *Acacia dealbata* and *Olea europaea*. *Antibiotics* 2020; **9(2)**, 48.
- [44] S Klongdee and U Klinkesorn. Optimization of accelerated aqueous ethanol extraction to obtain a polyphenol-rich crude extract from rambutan (*Nephelium lappaceum* L.) peel as natural antioxidant. *Scientific Reports* 2022; **12(1)**, 21153.
- [45] J Dai and RJ Mumper. Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules* 2010; **15(10)**, 7313-7352.
- [46] Y Niu and Y Xiang. An overview of bio membrane functions in plant responses to high-temperature stress. *Frontiers in Plant Science* 2018; **9**, 915.
- [47] I Izirwan, TD Munusamy, NH Hamidi and SZ Sulaiman. Optimization of microwave-assisted extraction of anthocyanin from *Clitoria Ternatea* flowers. *International Journal of Mechanical Engineering and Robotics* 2020; **9(9)**, 1246-1252.
- [48] WP Silvestre, GF Pauletti and C Baldasso. Fodder radish (*Raphanus sativus* L.) seed cake as a feedstock for pyrolysis. *Industrial Crops and Products* 2020; **154**, 1-12.
- [49] A Barone, J Glassey and G Montague. Towards online near-infrared spectroscopy to optimize food

- product mixing. *Journal of Food Engineering* 2019; **256**, 227-236.
- [50] FE Vasile, S Simal, C Rosselló and VS Eim. Power ultrasound-assisted impregnation of apple cubes with vitamin B₁₂. *Food and Bioprocess Technology* 2022; **15(1)**, 219-229.
- [51] P Wang, X Zhao, R Yang, Y Zhou, Q Zhou, Z Gu and D Jiang. Time-dependent extraction efficiency of betalains from *Beta vulgaris*. *Journal of Agricultural and Food Chemistry* 2020; **68**, 3784-3792.
- [52] P Suwanchaikasem, A Idnurm, J Selby-Pham, R Walker and BA Boughton. Root-TRAPR: A modular plant growth device to visualize root development and monitor growth parameters, as applied to an elicitor response of *Cannabis sativa*. *Plant Methods* 2022; **18(1)**, 46.
- [53] R Tanaka and K Nakamura. UV-vis spectral characteristics of carotenoids in *Capsicum annum*. *Phytochemical Analysis* 2019; **30(5)**, 567-573.
- [54] P Boonsong, N Laohakunjit, O Kerdehoechuen and FB Matta. Detection of pigments and natural colorants from Thai herbal plants for possible use as coloring dyes. *HortScience* 2011; **46(2)**, 265-272.
- [55] J Loum, R Byamukama and PAG Wanyama. Adsorption-kinetic studies of mordant exhaust dyeing of cotton and silk fabrics with dye extract of *M. lucida* plant species. *South African Journal of Chemistry* 2025; **75(1)**, 18-25.
- [56] M Nikita, B Niharika, T Milan, L Mousam and B Anup. Antimicrobial activity and phytochemical screening of traditional medicinal plants. *International Journal of Applied Sciences and Biotechnology* 2023; **11**, 186-196.
- [57] AB Sati and J Chandra. Application and optimization of natural mordants in modern dyeing. *Environmental Science: An Indian Journal* 2021; **8**, 253-256.
- [58] K Saba, A Masood and MS Khan. Multifunctional analysis of banana leaves extracts for dyeing. *Natural Product Communications* 2024; **19(2)**, 1-15.
- [59] MN Indira, J Sandra and AS Aleena. Impact of mordants on fabrics dyed with natural dyes. *African Journal of Biological Sciences* 2024; **6**, 4105-4115.
- [60] A Jyoti, A Prerna and G Gunjan. Rainbow of natural dyes on textiles using plant extracts: Sustainable and eco-friendly processes. *Green and Sustainable Chemistry* 2017; **7**, 35-47.
- [61] TG Kalyani and M Rafeekher. A comprehensive review on extraction techniques for natural dyes from flowers. *International Journal of Advanced Biochemistry Research* 2025; **9**, 403-407.
- [62] J Liu and MW van Iersel. Photosynthetic physiology of blue, green, and red light: Light intensity effects and underlying mechanisms. *Frontiers in Plant Science* 2021; **12**, 1-14.
- [63] P Kampeerappun, T Phattararittigul, S Jittrong, and D Kullachod. Effect of chitosan and mordants on dyeability of cotton fabrics with *Ruellia tuberosa* Linn. *Chiang Mai Journal of Science* 2010; **38(1)**, 95-104.
- [64] OS Nonso, O Genevieve, O Chinedu, N Onyinyechi and OC Kenneth. Effect of temperature and mordant on the dyeing of cotton using sodium hydroxide extract of *Whitfieldia lateritia* dye. *International Journal of Innovative Research in Science, Engineering and Technology* 2019; **8(6)**, 7301-7308.
- [65] W Chen, E Karangwa, J Yu, S Xia, B Feng and X Zhang. Effect of sodium chloride concentration on off-flavor removal correlated to glucosinolate degradation and red radish anthocyanin stability. *Journal of Food Science and Technology* 2019; **56(2)**, 937-950.
- [66] A Petjukevičs, A Batjuka and N Škute. The impact of different levels of sodium chloride on the quantitative changes of chlorophyll and carotenoids in chloroplasts of *Elodea canadensis* (Michx. 1803). *Biologija* 2015; **61(1)**, 34-41.
- [67] CC Nievola, CP Carvalho, V Carvalho and E Rodrigues. Rapid responses of plants to temperature changes. *Temperature Multidisciplinary Biomedical Journal* 2017; **4(4)**, 371-405.
- [68] I Smerj. Brief note on significance of absorption spectra in plant biology. *Journal of Plant Biochemistry and Physiology* 2023; **11**, 1-2.
- [69] A Rehman, M Irfan, A Hameed, MJ Saif, MA Qayyum and T Farooq. Chemical-free dyeing of

cotton with functional natural dye: A pollution-free and cleaner production approach. *Frontiers in Environmental Science* 2022; **10**, 848245.

[70] V Kumar and R Prabha. Extraction and analysis of natural dye. *Journal of Natural Products and Plant Resources* 2018; **8(2)**, 32-38.