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Optimization of Aqueous Extraction Method for Natural Dye from *Basella alba* Linn. Fruits: UV-Vis Analysis and FTIR Characterization

*Jan Alvin P. Daguplo, Kenneth L. Ciudad**

Department of Chemistry, College of Mathematics and Natural Sciences,
Caraga State University, Ampayon, Butuan City, Philippines

ABSTRACT

Natural dyes are valued for their biodegradability, non-toxic nature, and environmental sustainability. This study optimized an aqueous extraction method for dye obtained from *Basella alba* Linn. fruits, using water as the solvent, to develop a simple, safe, and cost-effective extraction approach. The extraction time and temperature were optimized based on UV-Vis spectroscopy analysis, while the effect on the dye was evaluated using Fourier Transform Infrared Spectroscopy (FTIR). The dye exhibited a λmax at 542nm, consistent with anthocyanin pigments, particularly malvidin. Absorbance increased with extraction time and temperature, with the highest value (1.56) recorded at 45 minutes and 90°C. The dye showed significant pH-dependent color changes, shifting from red (acidic) to purple and blue (neutral-alkaline). FTIR spectra at different pH values displayed only water-derived O-H and H-O-H bands, attributed to the low concentration of dye compounds resulting from the fruit's high moisture content (82.5%). Lake pigments prepared using lime displayed poor stability under light exposure. Integrating findings from previous studies, this work addresses a critical gap by defining optimized aqueous extraction conditions for *B. alba*, thereby supporting future applications in the development of textile, cosmetic, and food colorants.

Keywords: *Natural dye, Basella alba Linn., aqueous extraction, UV-Vis analysis spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), anthocyanins*

*Corresponding Author
*Email:klciudad@carsu.edu.ph
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1 Introduction

Dyeing has been considered an art form since ancient times, and natural dyes have been used for dyeing fabrics and apparel products (Ramona et al. 2014). However, the widespread availability and lower cost of synthetic dyes since their development in 1856 have led to a depletion in natural dyes (Samanta and Agarwal 2009). Moreover, the production of synthetic dyes has led to environmental pollution due to their use of toxic substances in production and the discharge of effluent after imparting color, leading to carcinogenic and allergic effects (Teklay and Kechi 2017). The production of synthetic dyes also involves non-renewable petroleum, and effluent discharge during application affects aquatic life (Berhanu and Ratnapandian 2017). As a result, there has been an increased demand for natural dyes over the past few decades (Teklay and Kechi 2017), as they offer several advantages, such as non-toxic functions, biodegradability, eco-friendliness, and even curative effects, such as curcumin's antibacterial properties. Natural dyes are found in nature, such as in plants, particularly in roots, barks, leaves, flowers, and fruits, and they are used to color textile goods such as cotton (Zamri et al. 2016). Among plant-derived natural dyes, anthocyanins are particularly attractive due to their vivid coloration and potential for use in textile, food, and cosmetic industries.

Basella alba Linn., commonly known as "Alugbati," contains anthocyanins and flavonoids, particularly in its ripe fruits, among other active components, that impart deep purplish-red coloration. These components have been shown to produce stains comparable to synthetic stains like crystal violet and safranin, and can be used as an alternative microbiological stain. However, limited research has examined the optimization of water-based extraction conditions for maximizing dye

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yield while maintaining pigment stability.

A study by Banias & Ciudad (2023) examined dye extracts and lake pigments from five plant species—including *Basella alba*—using water extraction and alum treatment. Their findings confirmed that *B. alba* fruits contain anthocyanin-based pigments but also revealed limited red intensity of lake pigments, poor pigment stability, and dominance of water solvent peaks in FTIR, indicating low extract concentration. Moreover, their extraction conditions were not optimized, as methods involved fixed parameters (4-hour heating, mordant presence) intended for pigment generation rather than maximizing dye yield.

Building on these findings that highlighted the limited stability and non-optimized extraction conditions of *Basella alba* pigments, this study seeks to establish an improved and systematic aqueous extraction method for *B. alba* fruits. Specifically, this research aims to: (1) determine the moisture content of the fruits using the gravimetric method; (2) optimize extraction time and temperature at a constant solid-to-liquid ratio to maximize dye yield; (3) evaluate the absorbance profile of the extract through UV-Vis spectrophotometry; (4) characterize pH-dependent structural behavior using Fourier Transform Infrared Spectroscopy (FTIR); and (5) produce lake pigments through adsorption on lime.

By eliminating the use of mordants during extraction and relying solely on water as a solvent, this study emphasizes a safe, cost-effective, and food-compatible approach to natural dye extraction. Its significance lies in generating foundational data that enhances pigment concentration, clarifies pH-responsive behavior, and supports future stabilization and formulation efforts for natural dye applications. The scope of this investigation is limited to ripe *Basella alba* fruits, which are abundantly available, and were collected in Butuan City, focusing exclusively on variations in aqueous extraction conditions. Nonetheless, the resulting optimized method and preliminary pigment production offer valuable insights for developing safer natural colorants suitable for potential textile, food, and cosmetic uses.

2 Materials and Methods

Collection and Preparation of Plant Samples

Fresh Alugbati fruits (Figure 1) were collected from multiple sites in Butuan City. Plant identity was confirmed by a taxonomist and validated by the local herbarium. The collected fruits were washed with running tap water to remove dust particles and rinsed with distilled water. A portion of the sample was set aside for analysis of its moisture content.

Determination of Moisture Content

Approximately one gram of fresh fruit samples were labeled "Wet Mass". These samples were oven-dried at 110 ± 5°C to a constant mass and labeled "Dry Mass". Three replicates were performed for

Figure 1. *Basella alba* Linn plant with ripe fruits

this analysis, and the percent moisture content was calculated as follows:

$$\%\text{Moisture} = \frac{\text{Wet mass} - \text{Dry Mass}}{\text{Mass of Wet Sample}} \text{x}100$$

It is important to note that the oven-drying procedure was conducted solely for the determination of moisture content and was not part of the extraction process, but was conducted to determine the actual solids available for extraction and to aid in interpreting the low concentration of analytes in the aqueous extract.

Extraction of Natural Dye

A 1:5 solid-to-liquid ratio (fruit to distilled water) was used. Extraction times (15, 30, 45, and 60 minutes) and temperature (30°C, 50°C, 70°C, and 90°C) were varied to produce 16 dye solutions, as shown in Table 1.

Table 1. Matrix for the dye solutions prepared from various extraction time and temperature

Solution Number	Time, min.	Temperature, °C
1	15	30
2	15	50
3	15	70
4	15	90
5	30	30
6	30	50
7	30	70
8	30	90
9	45	30
10	45	50
11	45	70
12	45	90
13	60	30
14	60	50
15	60	70
16	60	90

UV-Vis Analysis

For the initial determination of the λmax of the *Basella alba* dye, a representative extract was prepared following the aqueous extraction procedure described by Banias and Ciudad (2023), using the mordant-free variant of the method. Briefly, 100 g of ripe *B. alba* fruits were mixed with 500 mL of distilled water and heated at 80 °C for 4 h on a hot plate. The resulting solution was filtered through cloth to remove solids, and an aliquot of the filtrate was subjected to UV–Vis scanning from 250 nm to 750 nm at 2 nm increments, using a Perkin Elmer Ensight™ Multimode Microplate Reader UV-Vis Spectrophotometer at the Material Science and Polymer Chemistry Laboratory at Caraga State University.

The maximum absorbance was subsequently used as the analytical wavelength for comparing the different extraction time–temperature combinations tested in this study.

Effect of Varying pH

An aliquot of the dye solution (optimum extraction) was adjusted to pH 3, 5, 7, 9, and 11 using 1.0 M acetic acid and 0.5 M NaOH. Color changes were recorded. Attenuated Total Reflectance (ATR) FTIR was used to assess functional groups across pH-adjusted samples at the Material Science and Polymer Chemistry Laboratory.

Preparation of Lake Pigments by Adsorption on Lime

Dye solution (100 mL) was heated with ten grams of alum (aluminum potassium sulfate) at 70-90°C. The solution was filtered to remove residue and warmed at 50-60°C while slowly adding five grams of powdered lime. The lake pigment was then recovered from the supernatant liquid by filtration and air-dried.

3 Results and Discussion

Optimization of Extraction Time and Temperature

Distilled water was used as a solvent to extract the natural dye. Figure 2 shows the UV–Vis analysis of the dye solution over the 250–750 nm wavelength range at 2 nm increments, revealing a λmax at 542 nm. This indicates the presence of anthocyanin, particularly malvidin, responsible for colors in the red-blue range, consistent with malvidin (Wahyuningsih 2017).

Extraction time was varied at 15, 30, 45, and 60 minutes, while extraction temperature was set at 30, 50, 70, and 90 °C. The resulting absorbance values at 542 nm were then plotted against the corresponding extraction times and temperatures, as presented in Figure 3.

The solution extracted at 30 °C for 15 minutes had the lowest absorbance of 0.0931 at 542 nm. Absorbance increased with both time and temperature. A significant increase in absorbance occurred after 45 minutes of heating, and at 70 °C, the absorbance was already comparable to that of solutions heated for 60 minutes. The absorbance

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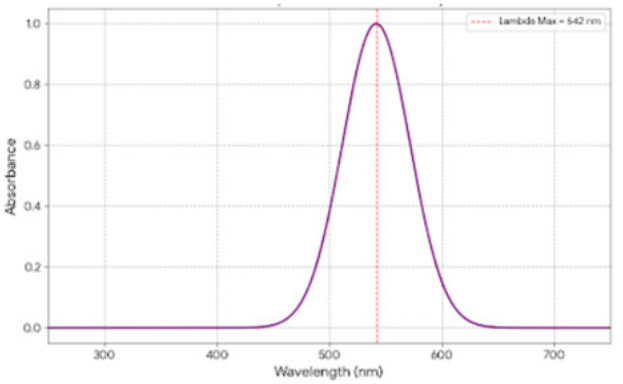

Figure 2. UV-VIS spectrum of *B. alba* dye solution

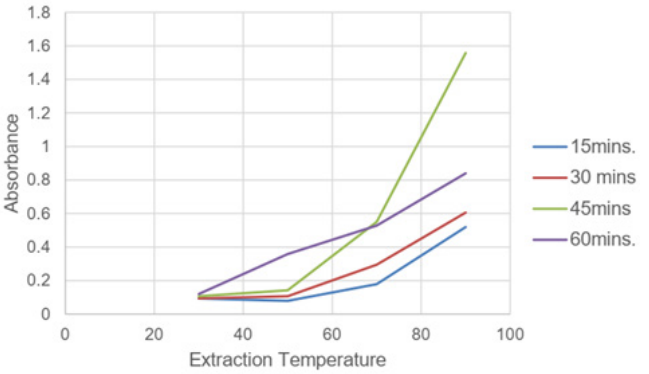

Figure 3. Effect of extraction time and temperature on the absorbance of the Alugbati dye solution at 542nm

continued to increase up to 1.56 at 90 °C. This trend indicates an increase in the concentration of extracted anthocyanins as reflected by the higher absorbance values.

The optimum extraction conditions were 45 minutes at 90 °C, producing a dye solution with the highest absorbance of 1.56. At lower extraction temperatures, diffusion and solvent penetration occur more slowly, requiring longer extraction times—such as 60 minutes—to effectively break down cell structures and release anthocyanins. However, at higher temperatures, improved solvent mobility, faster solute diffusion, and enhanced cell wall softening accelerate pigment release (Cacace & Mazza, 2003; Patras et al. 2010; Lapornik et al. 2005; Slavu et al. 2020), enabling the extraction to reach its maximum efficiency at 45 minutes. Prolonged heating beyond this point or extending exposure—particularly above 90 °C or past 60 minutes—may contribute to thermal degradation (Slavu et al. 2020), although this was outside the study's operational limits.

Effect of Varying pH on Dye Color

The initial pH of the dye solution extracted using the optimum extraction time and temperature was 5.00. Adjusting the pH of the solution affected its hue, with the color becoming lighter as pH decreased and darker as it approached neutrality or alkalinity as shown in Figure 4.

The stability of anthocyanin, influenced by factors such as pH, temperature, light, and oxygen, causes changes in color. Anthocyanins are red in acidic solutions, violet or purple in neutral solutions, and blue in alkaline pH. Most colorants containing anthocyanins can only be used at pH values below four, which is the stable condition of anthocyanin in the food and cosmetic industry (Wahyuningsih 2017).

At low pH, anthocyanin only protonates to form a positive ion or cation, while as pH increases, the molecules become deprotonated to form a negative ion or anion. Dye color shifted from red (pH 3) to purple (pH 7) to blue (pH 11). This behavior reflects anthocyanin structural transitions (flavylium

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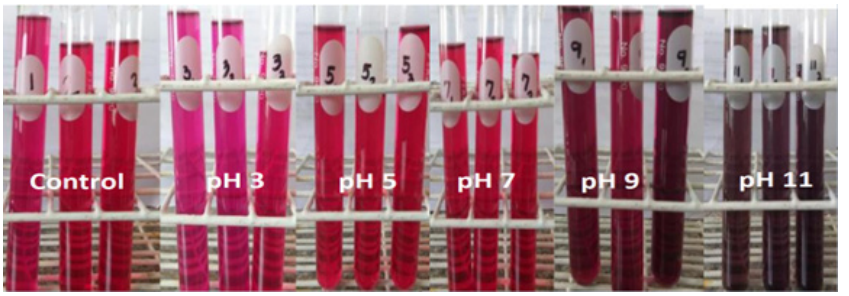

Figure 4. Color change observed in various pH extracted dyes for 45 minutes at 90°C from *Basella alba* Linn.

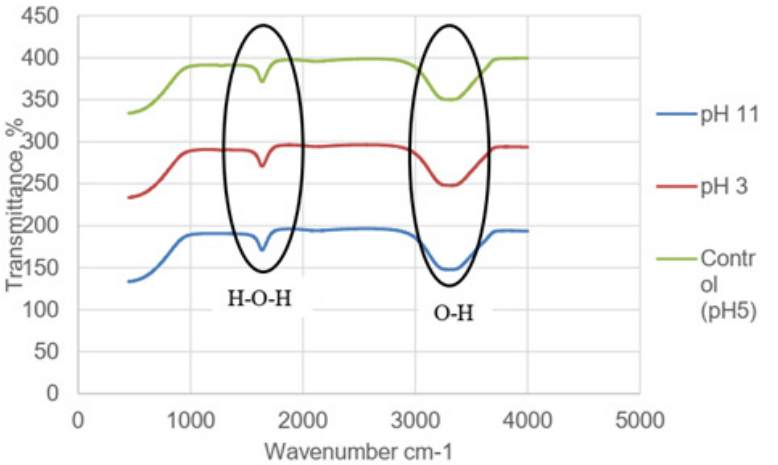

Figure 5. A plot of wave number vs. adjusted transmittance of natural dye at different pH

cation → quinonoidal base → anionic form). This finding aligns with Banias & Ciudad (2023), who also observed FTIR solvent dominance when using aqueous extraction.

FTIR Analysis

FT-IR analysis was performed to detect changes in the functional groups of the dye components resulting from varying pH levels. However, no differences were observed in the spectra of solutions with pH 3.00, 5.00, and 11.00. The spectra of these solutions contained only two peaks, corresponding to the broad and strong O-H stretching at 3200-3550 cm-1 and the H-O-H scissoring stretching at 1630-1780 cm^{-1}, as shown in Figure 5.

This suggests that hydroxyl groups associated with water dominate the spectra, masking other functional groups present in the extract, despite changes in pH. These match the spectrum of pure water, indicating that the dye concentration was too low to detect anthocyanin peaks; the solvent's strong absorbance overwhelmed the analyte peaks; and the high moisture content (82.5%) diluted the extract significantly.

Moisture Content Analysis

The moisture content analysis provides essential information regarding the solids available for extraction and helps explain the low concentration of detectable plant compounds in aqueous solutions. The individual moisture content values obtained from the three trials are summarized in Table 2, showing an average fruit moisture content of 82.50%.

Pigment Production

Dyes are converted into pigments for stability and permanence of the desired color properties. The Alugbati dye solution, prepared under optimum extraction conditions, was adsorbed onto lime powder to produce lake pigments, resulting in a pink pigment. However, the lake pigment faded rapidly with exposure to light, turning gray as shown in Figure 6.

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Table 2. Moisture Content of *Basella alba* Fruits

Trial	Wet mass (fresh weight, g)	Dry mass (g)	% Moisture	Average % Moisture (± SD)
1	1.0310	0.1440	86.032	
2	1.0650	0.2330	78.125	**82.50 (± 4.020)**
3	1.0930	0.1820	83.343	

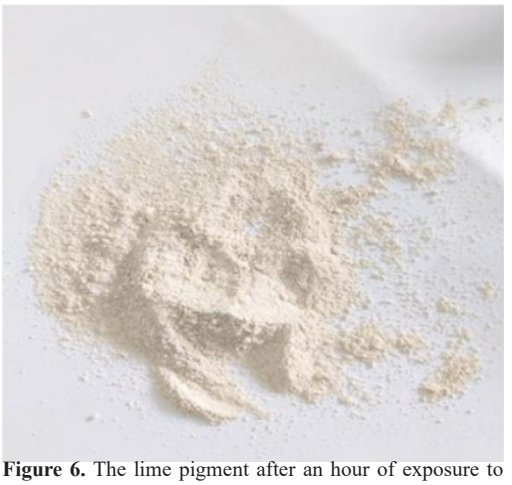

Figure 6. The lime pigment after an hour of exposure to ambient light

This could be due to the instability of anthocyanin pigments under light and oxygen (Wahyuningsih 2017). The instability mirrors earlier findings and supports recommendations for stabilization using co-pigmentation, encapsulation, or metal complexation. Further studies can be done to improve the stability of the pigments, such as incorporating stabilizing agents or using protective coatings.

4 Conclusion and Recommendations

The study demonstrated that the natural dye solutions extracted from *Basella alba* Linn. fruits have an absorption maximum at 542 nm, which corresponds to malvidin, an anthocyanin. The optimal extraction conditions were determined to be a 45-minute extraction time at 90°C, with an absorbance of 1.56. However, the pigment exhibits sensitivity to environmental factors such as pH, temperature, and light, and therefore, stabilization of the extracted dye is necessary to maintain its color.

The lack of distinct peaks in the FTIR spectra across different pH values reflects the limitations of the method due to the low concentration of anthocyanins and the dominant absorbance of water. Anthocyanins undergo well-established structural transformations in response to pH, temperature, and light, which explains the observable color changes despite the absence of detectable spectral differences. Future studies should increase the dye concentration or apply alternative analytical approaches to better resolve these structural shifts. Additionally, pigment stabilization strategies—through co-pigmentation, encapsulation, or the selection of plant materials with inherently more stable anthocyanins—are recommended to improve the color stability of Basella alba extracts. Nonetheless, the study was also limited by the absence of an anthocyanin standard for absolute concentration determination; thus, optimization was based on relative absorbance.

Further studies on the effect of moisture content on the dye solutions are recommended, as the high moisture content of 82.5% in the plant extracts may have contributed to the low concentration of the plant components. Additionally, the study suggests exploring using other mineral sources or natural powder-pigments with sorption capacity to improve the stability of the extracted dye. Encapsulation techniques should also be identified to maintain the color strength and coloring difference of the dye and pigment.

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5 Acknowledgment

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