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Optimization of Anthocyanin Extraction Process in Pacar Air (*Impatiens balsamina*) Flower from Canang Sari Flower Waste

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ABSTRACT. Canang sari is one of the offerings used in Hindu worship in Bali, consisting of various types of flowers, one of which is Impatiens balsamina Currently, the waste of canang sari flowers has not been utilized optimally. Pacar air (*Impatiens balsamina*) flower waste contains anthocyanin compounds and gives a purplish red color, which is promising as a natural dye raw material. This research aims to conduct phytochemical tests and determine the optimum operating conditions for extracting natural dyes from Pacar air (*I. balsamina*) flowers obtained from canang sari. The extraction of *I. balsamina* flowers uses acetone and ethanol solvents. Phytochemical, gravimetric, and UV-VIS spectrophotometric tests were conducted to determine the chemical content and stability. The extraction process is optimized using the Response Surface Methodology with Central Composite Design (CCD) type. The independent variables in this extraction process are: pH 1 – 4, temperature 40 – 70 °C, and extraction time 5 – 60 minutes, while the dependent or response variable is anthocyanin content. The control variables of this extraction process are the ratio of materials and solvents of 0.0625 g/mL, and the stirring speed of 300 rpm. As a result, the optimum operating conditions of the extraction process are pH 2.84, temperature 63.85 °C, and time 16.15 minutes, with anthocyanin levels of 18.05 mg/L.

INTRODUCTION

Flower waste generated from the canang sari consists of various types of flowers. Flowers are vital in canang as a spiritual symbol and tribute to the gods in Balinese Hinduism. The use of flowers in Bali is a basic necessity due to the culture and habits of the Balinese people, who make daily offerings in the form of canang sari. Therefore, a large volume of flower waste is left unattended and pollutes the surrounding environment after daily prayers and ceremonies on certain days. The flower of Pacar air (Impatiens balsamina) is an ornamental plant used to make canang. Pacar air plant (*I. Balsamina*) is a plant from the *Balsaminaceae* family that is very easy to grow in the home garden in the northern hemisphere, India, and mainland Southeast Asia, including Indonesia (Bole *et al*., 2014). Every part of this plant has many benefits, including the roots, stems, leaves, flowers, and fruits. Pacar air (*I. balsamina*) flower has a variety of colors, including red, pink, yellow, white, and purple. Pacar air (*I. balsamina*)has great potential as a natural dye. When used as a natural dye, Pacar air plant (*I. balsamina*) produces brownish or purple-red pigments.

Natural dyes are a renewable and sustainable bioresource product with a low environmental impact and have been used since ancient times, not only as textile dyes but also as food colors (Shahid *et al*., 2013). The source of raw materials for natural dyes comes from various types of biodiversity, which Indonesia has in abundance. Natural dyes can be produced through an extraction process. Extraction is the process of separating one or more substances from their mixture using a solvent, which must be able to extract the desired substance without dissolving other materials. Several factors, including the type of solvent, the ratio of raw materials to solvent, the size of the raw materials, the extraction temperature, the extraction time, the pH, and the extraction method, generally influence the extraction process.

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Some of the compounds involved in providing color pigments in Pacar air (*I. balsamina*) flowers are flavonols, which are a subgroup of flavonoids that can give flowers a yellow or white color; flavan-3-ol, which is another group of flavonoid compounds that can give color to flowers; and carotenoids, which are pigments that provide various plants their yellow, orange or red colour. While Pacar air (*I. balsamina*) flowers are commonly known for their blue or purple color, some variations or mutations can produce flowers of different colors, including yellow or orange, possibly due to carotenoids. Although several of these compounds can give Pacar air (*I. balsamina*) flowers their color, anthocyanins remain the primary compound responsible for the flower's most striking color. Anthocyanins are natural pigments in various plants that give flowers, fruits, and leaves their red, purple, or blue color. Anthocyanins are classified as water-soluble flavonoid pigments and are one of the primary pigment groups in higher plants (Wang *et al*., 2012). Anthocyanin pigments are found in the vacuoles of plant cells and are flavonoids that are naturally glycosides of flavylium or 2-phenylbenzopyrilium. This pigment is found in benzopyran derivatives (Nurtiana, 2019).

Anthocyanins have an aromatic hydrocarbon ring core comprising six carbon atoms forming a benzene structure. These carbon atoms are linked by single and double bonds, creating a stable aromatic ring. Several hydroxyl (OH) groups are possibly attached to certain carbon atoms in the benzene ring. The number and position of these hydroxyl groups vary depending on the type of anthocyanin present. These hydroxyl groups play a role in anthocyanins' chemical properties and reactivity. The pyrylium or pyridinium ion is a heterocyclic nitrogen ring bonded to a benzene nucleus. This ring consists of five carbon atoms and one nitrogen atom. The nitrogen atom that gives the anthocyanin molecule a positive charge, thus making it belong to the class of cationic compounds (Priska *et al*., 2018). Some anthocyanins also have sugar groups attached to the pyrylium ring. These sugar groups affect the solubility of anthocyanins and play a role in the interaction between anthocyanins and proteins and other polyphenols. In addition, variations in anthocyanin structure occur in other functional groups, such as methoxyl groups (O–CH_3) or acyl groups (e.g., acyl glucose or acyl tartaric acid). These structural differences give anthocyanins different color variations and physical properties.

The solvent extraction method is one of the techniques used to separate or isolate certain compounds from a mixture based on differences in the solubility of these compounds in the solvent used. The basic principle of this method is that solvent-soluble compounds will be released from the mixture and concentrated in the solvent phase. The choice of solvent in this method plays a critical role. The most commonly used organic solvents in anthocyanin extraction are ethanol, methanol, acidified water, or acidified ethanol (Ragab *et al*., 2022).

The solvent used to optimize the dye extraction process is ethanol. Ethanol or ethyl alcohol is a chemical compound that is often used as a solvent. It has the molecular formula C_2H_5OH. Ethanol is one type of solvent that is often used to extract natural pigments from plants. Ethanol as a solvent produces higher yields than acetone solvents due to the difference in chemical structure between ethanol and acetone (Rahayuningsih *et al*., 2017). Ethanol contains hydroxyl groups (–OH) while acetone does not. Structurally, ethanol has the molecular formula CH_5OH, which describes the composition and arrangement of atoms in the molecule. This structure gives ethanol its distinctive chemical properties, including solubility in water and its ability to interact with other polar compounds. The polar nature of the hydroxyl group (–OH) allows ethanol to form hydrogen bonds with water and other polar compounds, which is important in solvent properties.

Anthocyanin is more stable in acidic conditions than in alkaline conditions, where the stability of the structure ranged from coloured to colorless. When the pH changes, the color stability of anthocyanins will also change (Sirait, 2019). Anthocyanins in acidic solutions that have a pH of 1 – 3 are in a form dominated by flavylium cations (AH^+). In this condition, the anthocyanins produced will be very colorful and very stable. The condition at pH 4 – 5 or greater than pH 402 will trigger deprotonisation or hydration of Flavium cations and the formation of carbinol and chalcone base compounds that cause colorless or degraded colors. However, the lower the pH value, even if the pH is close to one, the more stable the color of the resulting concentrate (Sembiring, 2013).

Temperature can affect the color stability and degradation rate of anthocyanins. Temperature positively affects the efficiency and rate of extraction. Some active components can be degraded by temperature when the temperature is too high. The results of research conducted by (Hidayah *et al*., 2014) stated that the higher the heating temperature, the lower the absorbance or colour stability of the anthocyanin pigment, the results of the study indicated that at a temperature of 40 – 50 °C the colour pigment stability of the anthocyanin occurred and then at a temperature of 60 – 80 °C a decrease in the colour pigment produced. The reduction in colour stability is due to damage to the chromophore group of the pigment, which causes colour damage. According to Markakis

(1982) in (Lydia *et al*., 2001), the decrease in colour stability due to high temperatures is thought to be due to the decomposition of anthocyanins from aglycone to chalcone (colourless) form.

The amount of extracted compounds can be optimized by increasing the extraction time, but there are risks related to the degradation of thermolabile or destructible components (Damayanti *et al*., 2020). The adequate extraction time can affect the solvent's contact time with the sample to obtain a high colour pigment content (Sudarmi *et al*., 2015). Varying the time period is required for the extraction of various materials. However, exposure to a few seconds has shown promising results; besides that, the solvent's dielectric properties also affect the optimization of the extraction time (Damayanti *et al*., 2020). The longer the extraction time, the longer the contact time between the solvent and the material will be, so that from both there will be mass deposition by diffusion until the balance of solution concentration inside and outside the extraction material occurs (Wahyuni and Widjanarko, 2015).

The pH differential method determines the anthocyanin content in the sample. This method has been widely used by food and horticultural technologists to assess the quality of fresh and processed fruits and vegetables (Lee *et al*., 2005). This method takes advantage of the colour-changing properties of anthocyanins at different pH levels based on structural changes of anthocyanin chromophores between pH 1.0 and pH 4.5. Anthocyanin monomers undergo a structural reversible transformation as a function of pH (coloured oxonium form at pH 1.0 and colourless hemiketal form at pH 4.5). Thus, the difference in absorbance at the vis-max wavelength of 520 nm of the pigment is proportional to the pigment concentration. Anthocyanins that are degraded in polymeric form are resistant to discolouration with changes in pH (Lee *et al*., 2005). The absorbance should be measured at the vis-max of the pigment solution, and the pigment content should be calculated using its molecular weight (MW) and absorptivity coefficient expressed as cyanidin-3-glucoside. Therefore, this research aims to conduct phytochemical tests and determine the optimal operating conditions for extracting natural dyes from Impatiens balsamina flowers obtained from canang sari.

RESEARCH METHODS

The materials used include Pacar air (*I. balsamina*) flower, acetone (technical solvent), Ethanol 96%, Aquadest, Mayer reagent, Wagner reagent, Dragendorf reagent, Potassium chloride (KCl) Merck, Natrium acetate trihydrate ($CH_3COONa.\ 3H_2O$) (Smart-lab), Magnesium (Mg) Merck, Sodium hydroxide (NaOH) (Merck), Ferric chloride ($FeCl_3$) (Merck), Sucrose (Merck), Natrium chloride (NaCl) (Merck).

The tools used in the extraction process are soxhlet and reflux extraction tools. Other tools include UV-VIS spectrophotometry (Shimadzu UV mini 1240), oven (Memmert UF Series- UF110 Plus), desiccator, CIE Lab colourimeter (colourimeter WR10), beaker (Pyrex), petri dish, analytical balance (Ohaus PX224/E), pH meter (HI98103 Checker pH tester with 0,1 pH resolution- Hanna Instruments), hotplate and magnetic stirrer, filter paper.

Raw Material Preparation

Fresh Pacar air (*Impatiens balsamina*) flower raw materials were wind-dried for about 1 week without direct sun exposure and cut into smaller sizes.

Determination of Anthocyanin Content

Determination of anthocyanin levels in Pacar air (*I. balsamina*) flower waste was carried out using the Soxhlet method. Dried Pacar air (*I. balsamina*) flowers (15.625 g) were wrapped tightly using filter paper and put into a Soxhlet extraction tube. The solvent used was 250 mL of acetone. The extraction process was run until the solvent in the Soxhlet tube was clear after being allowed to stagnate for 1 night, as a sign that the natural dyes contained in the Pacar air (*I. balsamina*) flower waste had been extracted. After the solvent in the Soxhlet had cleared and the Soxhlet extraction process stopped, the solid sample was removed. Analysis of the determination of anthocyanin content in Pacar air (I. balsamina) flowers was carried out using the pH differential method. The extraction results (2.5 mL) were diluted in acetone. Furthermore, it is set at pH 1 and pH 4.5 respectively. After that, it was put into a cuvette to measure the absorbance using UV-VIS Spectrophotometry. The experiment of UV-Vis spectrophotometry absorbance measurement value was repeated 3 times. Measurements were made at wavelengths (λ) 520 nm and 700 nm.

Qualitative Phytochemical Screening Analysis

Alkaloid Test

Alkaloids were tested according to the Mayer, Wagner, and Dragendorff methods. Pacar Air flower extracts were placed in three different test tubes of 2 mL each, then 1 mL of 2 M HCl was added. Tube I was treated with 2-3 drops of Mayer's reagent, and a positive result was obtained if the solution formed a white precipitate. Tube II was filled with 2-3 drops of Wagner's reagent, and a positive result can be concluded if the solution formed an orange to brown precipitate. Dragendroff's reagent (2 – 3 drops) was added to tube III, and a positive result can be concluded if the solution forms an orange precipitate (Reiza *et al*., 2019).

Saponin Test

The saponin test was performed according to the Forth method, i.e., 2 mL of Pacar air flower extract was poured into a test tube, and then 10 mL of distilled water was added. The solution was then heated for 2 – 3 minutes and cooled down. Shake for 30 seconds. Observe the changes that occur. If a stable foam is formed (it does not disappear after 30 seconds), then the identification indicates the presence of saponins (Anggriani *et al*., 2017).

Flavonoid Test

The flavonoid test was performed according to the Shinoda method. A total of 0.50 mL of Pacar air flower extract was dripped onto the test tube. Then, three drops of methanol were added and stirred until homogeneous. After that, 0.1 g of magnesium powder was added, followed by three drops of concentrated HCl (Anggriani *et al*., 2017). The formation of yellow, orange, red, or blue colour indicates the presence of flavonoid compounds (Anggriani *et al*., 2017).

Phenolic Test

A total of 0.50 mL of the sample was dripped onto the test tube, then three drops of methanol were added and stirred until homogeneous, then three drops of $FeCl_3$ 5% were added. The formation of green, red, purple or blue colour indicates the presence of phenolic compounds (Anggriani *et al*., 2017).

Qualitative test for the detection of anthocyanins

A total of 0.5 mL of Pacar air flower extract was added to 2 M HCl and then heated at 100 ^{0}C for 5 minutes. The colour of the sample was then observed. If the red colour of the sample does not change, it indicates the presence of anthocyanins. NaOH 2M was added drop by drop. The colour changes from red to a blue-green colour which slowly fades indicates the presence of anthocyanins (Anggriani *et al*., 2017).

Determination of optimum conditions for the extraction process of anthocyanin content in Pacar air (Impatiens balsamina) flowers

In the extraction stage, the principle is to contact the raw material to be extracted with a solvent in an extractor. The independent variables in the extraction optimization stage of Pacar air flower waste are varying pH (1 – 4), temperature variations (40 – 70 ºC), and time configuration variations (5 – 60 minutes). The dependent variable to be known is the anthocyanin content in Pacar air flower waste, expressed by the anthocyanin content in total natural dye (ZWA) of Pacar air flower waste. Meanwhile, the control variables in this study are the ratio of raw materials to solvent of 15.625 g into 250 mL of ethanol solvent and the stirring speed of 300 rpm. The determination of anthocyanin levels in Pacar air flowers was carried out using the pH differential method. The extraction results, as much as 2.5 mL produced in the previous process, were diluted using acetone according to the dilution factor. Furthermore, the pH solution was set at pH 1 and pH 4.5. After that, the solution was put into a cuvette, and the absorbance was measured using UV-VIS Spectrophotometry at wavelengths (λ) 520 nm and 700 nm, and was repeated 3 times.

Data Analysis

UV-VIS Spectrophotometric test

The absorbance of each sample was measured at λ max and λ 700 nm. The extraction samples were tested for absorbance using a UV-Vis spectrophotometer at the maximum wavelength of 520 nm. Absorbance was calculated using Equation 1 (Arroy *et al*., 2017),

$$A = (\lambda_{520}-\lambda_{700})\ \text{pH 1} - (\lambda_{520}-\lambda_{700})\ \text{pH 4.5} \quad (1)$$

while the anthocyanin content was calculated according to Equation 2,

$$Anthocyanin\ content\ \left(\frac{mg}{l}\right) = \frac{A\ x\ BM\ x\ FP\ x\ 1000}{\varepsilon\ x\ L} \quad (2)$$

Where λ_{520} = maximum absorption of the sample, λ_{700} = absorption of cyanidin-3-glucoside, A = maximum value of absorption, ε = coefficient of absorption (26900 L/mol.cm) expressed in terms of cyanidin-3-glucoside, BM = molecular weight of cyanidin 3- glucoside (449.2 g/mol), FP = dilution factor (1), L = cuvette width (1 cm).

Response Surface Methodology

The experimental design used in this research was the Central Composite Design (CCD). This Central Composite Design (CCD) design is an experimental design that serves to determine the number of experiments to be used, which were then evaluated for response optimization and variables. The Central Composite Design (CCD) method has three points: factorial, axial, and central (Dwiastuti and Dewi, 2022). By using these three points, Central Composite Design (CCD) allows the formation of an accurate model and can be used to predict and optimise responses to various combinations of factors. Based on three independent variables that influence this study, the experimental design is presented in Table 1.

Table 1. Variable code level in research.

Variabel	Level				
	-1.68	-1	0	+1	+1.68
pH (X_1)	X_{1a}	X_{1b}	X_{1c}	X_{1d}	X_{1e}
Suhu Ekstraksi (X_2)	X_{2a}	X_{2b}	X_{2c}	X_{2d}	X_{2e}
Waktu Ekstraksi (X_3)	X_{3a}	X_{3b}	X_{3c}	X_{3d}	X_{3e}

RESULTS AND DISCUSSION

Content and Stability of Chemical Compounds in Pacar Air (*I. balsamina*) Flowers

To determine the content and stability of chemical substances contained in Pacar air (*Impatiens balsamina*) flowers, the preliminary tests of samples were carried out first on two conditions of Pacar air (*I. balsamina*) flowers, namely, fresh Pacar air flowers and wind-dried Pacar air flowers. As for some of the tests carried out in this preliminary test, namely:

Qualitative Phytochemical Screening

This test was conducted to qualitatively determine the chemical content in Pacar air (I. balsamina) flowers. The following are the results of phytochemical screening on fresh (Table 2) and wind-dried (Table 3) conditions of Pacar air (*I. Balsamina*) flowers:

Table 2. Qualitative phytochemical screening for fresh Pacar air (*I. balsamina*) flower.

Qualitative Phytochemical Screening		
Type of Test	**Result**	**Description**
Alkaloid Test		
1. Mayer's Reagent	+	White precipitate formed, although not very clear
2. Wagner's Reagent	+	Orange to brown precipitate formed
3. Dragendorff's Reagent	+	Orange precipitate formed
Saponin Test	++	Produces continuous foaming for up to 30 seconds
Flavonoid Test	+	Yellowish colour formed
Phenolic Test	+	Yellow-green colour formed
Qualitative test for the detection of anthocyanins		
1. With added HCl	++	Bright red colour of the sample remains unchanged
2. With added NaOH	++	Red colour changes to green which slowly fades

In these two raw material conditions, fresh and wind-dried Pacar air (I. balsamina) flowers have anthocyanin content, but there are differences in the red colour produced. Fresh Pacar air flowers produce a lighter red colour, while wind-dried Pacar air flowers produce a brownish red colour.

Table 3. Qualitative phytochemical screening for wind-dried Pacar air (*I. balsamina*) flowers.

Qualitative Phytochemical Screening		
Type of Test	**Result**	**Description**
Alkaloid Test		
1. Mayer's Reagent	++	Very clear white precipitate formed
2. Wagner's Reagent	++	Orange to brown precipitate formed
3. Dragendorff's Reagent	++	Orange precipitate formed
Saponin Test	+	Foam formed, but only up to 10 seconds
Flavonoid Test	++	Yellow to orange colour formed. There is a slight red colour.
Phenolic Test	++	Intense green and slightly yellowish colour formed
Qualitative test for the detection of anthocyanins		
1. With added HCl	++	The reddish-brown colour in the sample is not changing
2. With added NaOH	++	Red colour changes to a green colour that slowly fades

UV-Vis Spectrophotometry Test

The UV-Vis spectrophotometry test aims to analyse the overall anthocyanin content contained in Pacar air (*I. balsamina*) flowers. Table 4 shows the results of anthocyanin content using the UV-Vis spectrophotometry test on each condition of Pacar air (*I. balsamina*) flowers:

Table 4. Anthocyanin content of Pacar air in different treatment

Condition of The Sample	**Anthocyanin Content (mg/l)**
Fresh Pacar air (*I. balsamina*) Flower	20.44
Wind-dried Pacar air (*I. balsamina*) Flowers	10.22

Anthocyanin content in each condition of Pacar air (*I. balsamina*) flowers shows significant differences in results. Fresh Pacar air (*I. balsamina*) flowers produce higher anthocyanin content. This is due to several factors, namely:

Water content in the sample

Fresh flowers have a moisture content of 93.14%, which is higher than the moisture content of wind-dried flowers. Water has a function to help dissolve and stabilise anthocyanins during the extraction process, which leads to higher anthocyanin levels in fresh flower extracts compared to dried flowers. When flowers are dried, water is lost and the stability of anthocyanins decreases, making them more susceptible to oxidative damage and thermal degradation.

Drying Method and Time

The drying method used is wind drying, which often causes greater anthocyanin loss due to the slow process and longer exposure of the flowers to environmental conditions. Drying methods involving exposure to oxygen tend to increase oxidation levels and decrease humidity gradually. There is a slow water loss, which can lead to changes in pH and microenvironment in the flower and affect the cellular structure of the flower (Machado *et al*., 2023). In addition, drying time also has a major impact on anthocyanin content in flowers, as longer drying time can increase exposure to factors that cause degradation. During prolonged drying, physical changes such as shrinkage and mechanical damage can also affect the stability and anthocyanin content.

Oxygen Exposure and Drying Temperature

During the drying process, flowers are exposed to oxygen for a longer period of time, while anthocyanin pigments are very sensitive to oxygen, so this is also one of the factors that cause anthocyanins to degrade quickly. Oxygen can cause the oxidation of anthocyanins, which can change their chemical structure into colourless or less colourful or less stable forms. This oxidation involves the loss of electrons from the anthocyanin molecule to damage the bonds in its molecular structure (Zhang *et al*., 2023). Exposure to oxygen for a long duration can also produce free radicals that damage anthocyanin molecules; these free radicals are highly reactive in accelerating anthocyanin degradation.

Wind drying at room temperature is not low enough to prevent enzymatic activity and chemical reactions that can damage anthocyanins. Room temperature tends to favor optimal activity for many oxidative enzymes. These enzymes remain active and efficient in catalyzing oxidation reactions that damage anthocyanins over long drying periods (Zhang *et al*., 2023).

During drying, anthocyanins undergo degradation due to oxidation, free radical formation, enzymatic oxidation, and hydrolysis. The key chemical reactions describing these processes are as follows.

Oxidation of Anthocyanins

Anthocyanins (Acy) are susceptible to oxidation, leading to structural changes that result in color loss. The general oxidation reaction can be written as Reaction 1.

$$Acy + \frac{1}{2}O_2 \rightarrow Degraded\ Anthocyanin + H_2O_2 \quad \text{(Reaction 1)}$$

Hydrogen peroxide (H_2O_2) is a byproduct that accelerates further degradation.In conclusion, that oxygen exprosure leads to oxidation and degradation of anthocyanins.

Free Radical Formation

Oxygen exposure results in the formation of reactive oxygen species (ROS), which contribute to anthocyanins degradation as presented in Reactions 2 – 4.

$$O_2 + e^- \rightarrow O_2^- \text{ (Super Radical)} \quad \text{(Reaction 2)}$$

$$O_2^- + H_2O \rightarrow OH + OH^- \text{ (Hydroxyl Radical Formation)} \quad \text{(Reaction 3)}$$

$$OH + Acy \rightarrow Degraded\ Anthocyanin + Byproducts \quad \text{(Reaction 4)}$$

Free radicals such as hydroxyl radicals (OH) and superoxide radicals (O_2^-) attack anthocyanins, breaking their bonds and leading to discoloration. In conclusion, free radicals accelerate anthocyanin breakdown.

Enzymatic Oxidation (Polyphenol oxidase and Peroxidase)

Oxidative enzymes, such as polyphenol oxidase (PPO) (Reaction 5) and peroxidase (POD) (Reaction 6), catalyze anthocyanin oxidation in the presence of oxygen.

$$Acy + O_2 \xrightarrow{PPO} Quinone - like\ Compounds + H_2O_2 \quad \text{(Reaction 5)}$$

Quinone-like compounds contribute to browning and further pigment degradation.

$$Acy + O_2 \xrightarrow{POD} Degraded\ Products + H_2O \quad \text{(Reaction 6)}$$

Since peroxidase utilizes hydrogen peroxide, this reaction amplifies anthocyanin breakdown. In conclusion, Enzymatic activity (PPO, POD) catalyzes oxidation and degradation.

Hydrolysis and Structural Breakdown

At higher drying temperatures, anthocyanins degrade into chalcones and phenolic acids, causing loss of pigmentation as shown in Reaction 7.

$$Acy + H_2O \rightarrow Chalcone + Phenolic\ Acids \quad \text{(Reaction 7)}$$

This reaction is temperature-dependent and is promoted by prolonged drying at room temperature. Hydrolysis at room temperature further reduces pigment stability.

Colour Stability Test using Colorimeter

This test aims to analyse and ensure the colour consistency of a sample material during the production and storage process. In this test, sugar and salt were added. The second function of this test is to use an additional experimental tool that is useful for evaluating how environmental factors or certain materials affect the colour stability of a dye product. The instrument used to measure colour stability is a colorimeter. The colour system used in this colorimeter is Hunter's Lab Colorimetric System, Hunter's colour notation system was characterized by three values, namely L (Lightness or brightness), a* (Redness or redness level), and b* (yellowness or yellowish level) (Indrayati *et al*., 2013). L, a, b values have scale intervals that indicate the colour level of the material being tested. From the reading of the colourimeter tool, the values of the three will be obtained. This ΔE search aims to measure and evaluate the colour difference between two samples. In addition, another main function is to ensure colour consistency, monitor colour changes over time, and assess whether the colour difference is within acceptable tolerance limits. This is very important in product quality control, ensuring colour stability, and maintaining aesthetic standards in various industries. Equation 3 is the formula used to find ΔE (Mokrzycki and Tatol, 2014)

$$\Delta E_{Lab} = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (3)$$

This reading of the ΔE_{Lab} value will provide a quantitative value of the colour changes and differences experienced. The ΔE_{Lab} value is usually measured in units that have no units. The following is the standard used

to see the colour difference that occurs (Mokrzycki and Tatol, 2014): $\Delta E < 1$ indicates the colour is almost invisible to the human eye; $1 \leq \Delta E < 2$ indicates the colour change may be visible with careful observation; $2 \leq \Delta E < 5$ indicates the colour change is clearly visible; $\Delta E \geq 5$ indicates a very noticeable colour change.

Table 5 shows the results of the stability test on the extraction of Pacar air (*Impatiens balsamina*) flower in each sample condition:

Table 5. Stability test results on Fresh Pacar Air Flower extraction using a colorimeter.

ΔE lab	Day to-				
	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**
Sugar 3%	4.97	4.33	2.60	1.00	1.80
Sugar 6%	3.99	4.05	2.37	2.51	1.27
Sugar 10%	3.23	5.90	1,63	2.41	2.39
Salt 3%	2.65	3.03	1.80	2.06	1.23
Salt 6%	3.67	4.18	2.84	3.19	2.16
Salt 10%	3.97	4.91	1.72	2.71	2.44

This daily ΔE lab analysis aims to determine whether the colour change is stable, increasing, or decreasing. When viewed from the results of stability tests on both fresh and wind-dried Pacar air (*I. balsamina*) flower extractions using a colorimeter, for fresh Pacar air flowers the resulting ΔE lab range is between 2 - 4 indicating that there is a clearly visible colour change and for wind-dried Pacar air flowers the resulting ΔE lab range is between 1-2 indicating that there is a possible colour change that is visible with careful observation. In addition, in both circumstances, the trend of ΔE lab per day shows a decrease every day in the Pacar air (*I. balsamina*) flower extract against the addition of sugar and salt.

Optimisation of Anthocyanin Extraction Conditions from Pacar Air *(Impatiens balsamina*) Flower

The experimental design in this study uses Response Surface Methodology (RSM) with Response Surface Central Composite Design (CCD) by utilising the Design-Expert® 13 application. The treatment response that will be generated is anthocyanin levels (mg/L). After inputting the factor and response to the application, 20 experimental designs appear presented in Table 6. From these data, the Design Expert program obtained the following optimisation values, namely in the condition pH 2.84; temperature 63.85 °C; and time 16.15 minutes with anthocyanin content of 18.05 mg/L and desirability 1.00.

Table 6. Results of anthocyanin levels in the extraction process.

No	pH	Temperature (°C)	Time of extraction (minute)	Anthocyanin Content (mg/L)
1	1	55	32.5	6.61
2	1.6	46	16	5.08
3	1.6	46	49	3.34
4	1.6	64	16	9.48
5	1.6	64	49	5.08
6	2.5	40	32.5	27.79
7	2.5	55	5	30.52
8	2.5	55	32.5	23.78
9	2.5	55	32.5	29.59
10	2.5	55	32.5	24.18
11	2.5	55	32.5	25.18
12	2.5	55	32.5	20.71
13	2.5	55	32.5	20.44
14	2.5	55	60	12.96
15	2.5	70	32.5	13.49
16	3.4	46	16	27.52
17	3.4	46	49	18.57
18	3.4	64	16	9.22
19	3.4	64	49	6.41
20	4	55	32.5	10.89

The mathematical equation model generated and used to predict whether or not the anthocyanin content is significant depends on the probability value (p) analysed in ANOVA. If the p-value is <0.05, the variable can be said to have a significant effect on other variables. The smaller the p-value, the greater the significance level (Agustini *et al*., 2014). The ANOVA results obtained are presented in Table 7.

Table 7. ANOVA results on the quadratic model for p-value and F-value.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1371.75	9	152.42	5.88	0.0053	**significant**
A-pH	154.48	1	154.48	5.96	0.0347	
B-Suhu	171.20	1	171.20	6.61	0.0278	
C-Waktu	164.83	1	164.83	6.36	0.0302	
AB	167.48	1	167.48	6.47	0.0292	
AC	3.94	1	3.94	0.1519	0.7049	
BC	1.51	1	1.51	0.0582	0.8142	
A^2	638.87	1	638.87	24.67	0.0006	
B^2	86.83	1	86.83	3.35	0.0970	
C^2	61.45	1	61.45	2.37	0.1545	
Residual	258.99	10	25.90			
Lack of Fit	202.74	5	40.55	3.60	0.0929	**not significant**
Pure Error	56.25	5	11.25			
Cor Total	1630.74	19				

The ANOVA results for the quadratic model are shown in Table 7. The p-value obtained shows that each component, namely pH, temperature, and extraction time, has a real (significant) effect on the response of anthocyanin levels. The Lack of Fit F-value for the anthocyanin level response shows a p-value greater than 0.05 ($p > 0.05$), indicating that the Lack of Fit is not significant. This finding satisfies the requirement that a Lack of Fit p-value above 0.05 must be insignificant, confirming that the developed model is appropriate, as the anthocyanin response data aligns well with the model (Rahmawati *et al*., 2022). In ANOVA, the model p-value reflects the overall goodness of fit, while the Lack of Fit specifically evaluates the model's adequacy at a more detailed level. The quadratic Response Surface Methodology (RSM) equation for optimizing anthocyanin extraction conditions is presented in Equation 4:

$$Y = 24.17 + 3.36\,A - 3.54\,B - 3.47\,C - 4.58\,AB - 0.7014\,AC + 0.4342\,BC - 6.66\,A^2 - 2.45\,B^2 + 2.06\,C^2 \quad (4)$$

A is pH, B is temperature, and C is extraction time.

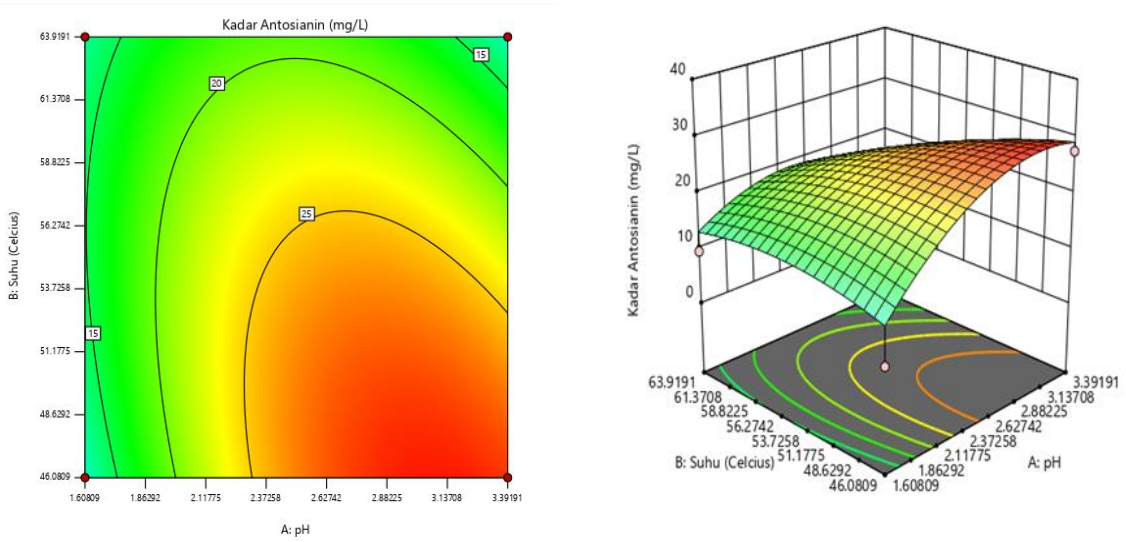
Analysis of anthocyanin content in response to the influence of pH and temperature of extraction

Visualisation of contour and three-dimensional plots of the response results of anthocyanin content influenced by pH and extraction temperature factors, contour conditions have anthocyanin content ranging in value from 15 – 25 mg/mL, showing a color difference, in which the higher the anthocyanin content, the redder the area, as shown in Figure 1a.

Based on Figure 1b, the resulting response's three-dimensional (3D) condition is a parabolic shape, where the quadratic equation used is a power of two. The results of the high anthocyanin content response are in the process conditions with a pH<3 and a relatively high temperature of around 56 °C, the area shown is yellow to red. At low pH (pH<3), anthocyanins are more stable in the form of flavylium cations, which are red in colour. This means that the colour of anthocyanins will tend to remain red even though it is affected by high temperatures, because this form of flavylium cation is stable in acidic conditions. However, although low pH can help maintain the stability of anthocyanins, relatively high extraction temperatures can still cause anthocyanin degradation. Therefore, the extraction temperature should be controlled not to be too high to ensure efficient extraction and avoid anthocyanin degradation.

Analysis of anthocyanin content in response to the effect of pH and time of extraction

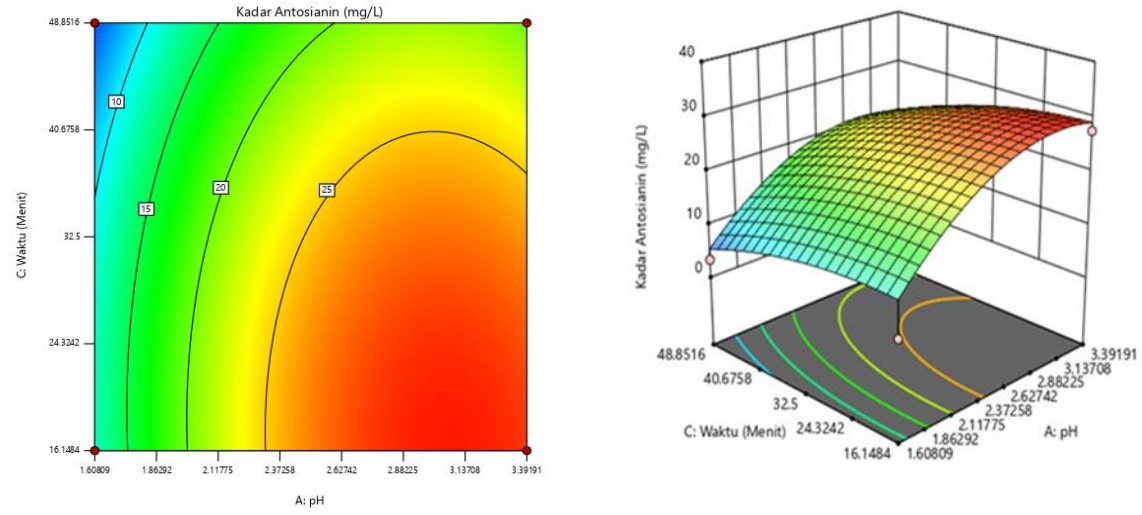
Figure 2 shows that the colour conditions displayed are blue and red, with blue areas representing areas with low anthocyanin content, while red ones show the results of high anthocyanin content. Figure 2a shows that contour lines correspond to the range of anthocyanin levels of 10 – 25 mg/L. The contour visualization shows that at pH 2.3–3.4, a bright red color is observed, indicating high anthocyanin levels. At pH values above 3, high anthocyanin content can also be obtained, but only within a shorter extraction time. The trend demonstrates that increasing pH tends to enhance anthocyanin levels, while longer extraction times generally decrease anthocyanin content.



(a) **(b)**

Figure 1. (a) Contour and (b) three-dimensional (3D) visualisation of anthocyanin content response with the effect of pH and temperature of extraction.

Specifically, at pH below 3 and extraction times under 40 minutes, anthocyanin stability is higher, resulting in greater pigment yield. Conversely, at pH values above 3 and with prolonged extraction, anthocyanins become more prone to degradation due to reduced stability. Therefore, optimizing extraction requires shorter processing times to minimize pigment loss. At acidic pH (below 3), anthocyanins predominantly exist as flavylium cations, which are stable and responsible for the intense red coloration. This structural form enhances resistance to degradation, provided the extraction time remains relatively short. Thus, maintaining a low pH and limiting extraction duration are key factors in preserving both the quality and quantity of anthocyanin pigments.



(a) **(b)**

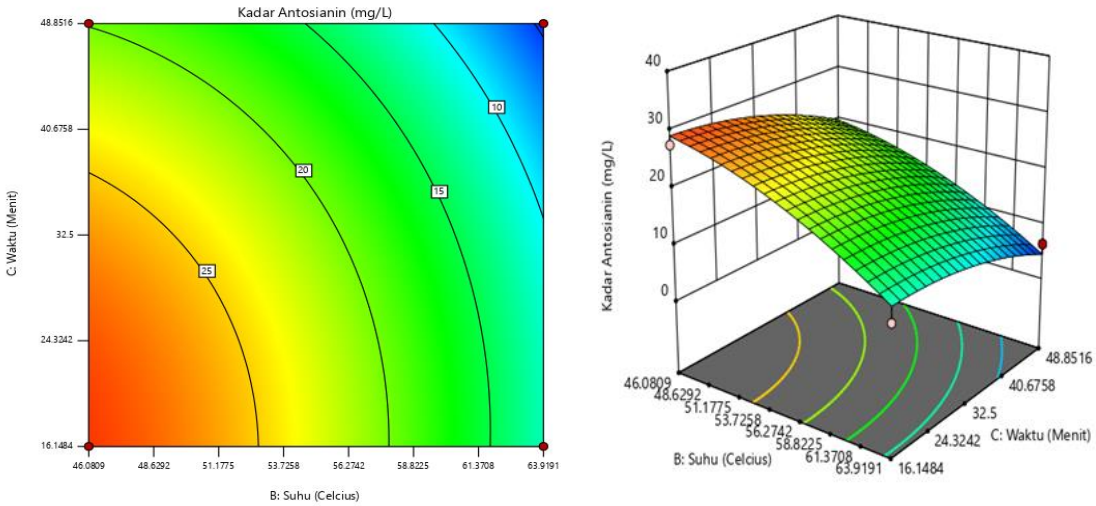
Figure 2. (a) Contour and (b) three-dimensional (3D) visualisation of anthocyanin content response with the effect of pH and time of extraction.

Analysis of anthocyanin content in response to the Effect of Temperature and Time of extraction

Visualisation of the contour plot of anthocyanin results influenced by temperature and extraction time factors shows contour lines ranging from 10 – 25 mg/L . The highest anthocyanin content is shown in red colour, which is influenced by the extraction temperature factor below 55 °C and extraction time below 40 minutes (Figure 3).

The temperature used in the extraction to produce optimum anthocyanin content is between 50 °C and 54 °C. This temperature is relatively high compared to the temperature commonly used to extract anthocyanins, which is around 40 – 50 °C. The anthocyanin yield can be significantly affected when the extraction temperature is relatively

high and the extraction time is relatively short. Relatively high temperatures result in anthocyanin extraction rates that tend to increase due to faster diffusion kinetics. High temperatures facilitate the release of anthocyanins from the cellular matrix of the plants used as raw materials, so the concentration of anthocyanins extracted in a short time can be higher. Using a relatively short or short extraction time can minimize the degradation effect of anthocyanins resulting from high temperatures. Short extraction times can provide a balance between maximizing the yield of stable anthocyanins and minimizing the damage caused by high temperatures. Exposure to high temperatures for long periods of time results in anthocyanin levels that tend to be unstable and can cause anthocyanin degradation into colourless or discoloured products. The optimum conditions for the natural dye extraction process were achieved at pH 2.84, a temperature of 63.85 °C, and an extraction time of 16.15 minutes, yielding an anthocyanin concentration of 18.05 mg/L.



(a) **(b)**

Figure 3. (a) Contour and (b) three-dimensional (3D) visualisation of anthocyanin content response with the influence of extraction temperature and time.

Colour stability under optimised conditions of the extraction process

This daily ΔE lab analysis aims to determine whether the colour change is stable, increasing, or decreasing, as shown in Table 8. Based on the stability test results at the optimum extraction conditions using a colorimeter, the results show a ΔE lab range between 7–14, indicating a striking colour change per day. In addition, the daily ΔE lab trend demonstrates instability (fluctuations of increase and decrease) in the Pacar air (Impatiens balsamina) flower extract when sugar and salt are added. This instability is influenced by the readings of brightness level (L), red colour level (a*), and yellow colour level (b*) obtained from the colorimeter with the addition of sugar and salt at different concentrations. Such variations in L, a*, and b* values occur because sugar and salt can affect the pH of the solution, its viscosity, water activity, and the solubility of anthocyanins in the extract..

Table 8. Results of colour stability test with colorimeter at optimum condition of Pacar air (*I. balsamina*) flower extraction process.

ΔE lab	Day to-			
	Day 1	**Day 2**	**Day 3**	**Day 4**
Sugar 3%	7.65	18.56	9.86	9.37
Sugar 6%	8.05	19.16	9.58	10.10
Sugar 10%	11.64	17.84	8.65	13.16
Salt 3%	15.97	13.28	6.50	12.61
Salt 6%	14.52	12.96	9.33	13.86
Salt 10%	13.06	15.14	8.18	7.42

CONCLUSION

Processing natural dyes using raw materials by Pacar air (*I. balsamina*) flowers, with two sample conditions, namely fresh and wind-dried Pacar air (*I. balsamina*) flowers, using the solvent extraction method in

separating the compound components contained in the sample. Phytochemistry and UV-Vis spectrophotometry were conducted to determine the chemical content and stability. Anthocyanin content in fresh and wind-dried Pacar air (*I. balsamina*) flower conditions was 20.4394 mg/L and 10.2197 mg/L. The optimum operating conditions of the natural dye extraction process are at pH 2.84, temperature 63.85 °C, and extraction time 16.15 minutes, resulting in anthocyanin levels of 18.05 mg/L.

CONFLICT OF INTEREST

There is no conflict of interest in this article.

AUTHOR CONTRIBUTION

ASF: Conceptualization, Methodology, Investigation, Software, Data Analysis, Writing – Original Draft, Manuscript Drafting, Editing; ER: Supervision, Conceptualization, Methodology, Data Analysis, Validation, Funding Acquisition, Manuscript Review and Editing; RBC: Supervision, Conceptualization.

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DECLARATION OF GENERATIVE AI

The authors acknowledge the use of AI-assisted tools (ChatGPT, OpenAI) for language editing purposes only.

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