Extraction of anthocyanin pigment from *hibiscus sabdariffa* L. by ultrasonic-assisted extraction

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Abstract. Roselle flower contains various active compounds including organic acid, hydroxycitric acid, hibiscus acid, anthocyanin, flavonoids and polysaccharides. The anthocyanin compounds in rosella flowers are the most important pigment of vascular plants, harmless and that make it interesting is a soluble pigment in water media. Anthocyanin extract is one of the most successful natural sensitizers for solar cells, because this organic dye produces a high yield of photons to current conversion and is easy to obtain and cheap. Anthocyanins can be potential as sensitizers, because they range in the light spectrum from red to blue. The purpose of this study was to obtain anthocyanin pigments at the optimum extraction conditions from roselle flowers. The optimization of the method was carried out by analyzing the effect of ultrasonic wave frequency, process temperature, extraction time, and mass ratio of roselle flowers to the volume of distilled water on the anthocyanin concentration. The qualitative and quantitative compositions of the anthocyanin extract of rosella flowers were analyzed by UV-Vis spectrophotometer. The results showed that the optimal operating conditions for the extraction process were known at a temperature of 40 °C, a frequency of 24 kHz, a time of 5 minutes, and a ratio of 1:25.

1. Introduction

One of the pigments that can be used as a sensitizer is anthocyanin. Anthocyanins can be obtained from various natural sources such as rosella flowers. Rosella flowers contain various active compounds, including organic acids, hydroxycitric acid, hibiscus acid, anthocyanins, flavonoids and polysaccharides [1–4]. The anthocyanin compounds in rosella flowers are the most important pigments of vascular plants, are harmless and what makes them attractive are pigments that are easily soluble in water [5]. Anthocyanin extract is one of the most successful natural sensitizers for solar cells, because this organic dye produces high conversion yields of photons to high currents and is easy to obtain and cheap [6]. Anthocyanins can be potential as sensitizers, because they range in the light spectrum from red to blue. Anthocyanins also have carbonyl and hydroxyl groups so that they can bind to the TiO₂ surface [7].

Anthocyanins are natural secondary metabolite compounds that give color to several types of fruits, vegetables and plants [8]. This pigment is classified as a flavonoid compound which has a function as a natural antioxidant. Antioxidants are substances that can ward off or prevent oxidation reactions from free radicals. This compound is soluble in water and is polar so that it can be dissolved in polar solvents such as ethanol, acetone, chlorophome, and water chloride or formic acid [8].
Anthocyanins are obtained from several sources such as eggplant [9], pomegranate [10], red cabbage [11], and strawberries. It was reported that the anthocyanins obtained from Rosella were delphinidin and cyanidin complexes. These functional groups contribute to the color stability of the roselle flower extract [12]. Rosella has many uses in food, but there are other uses such as use in solar cells. Roselle was chosen as a potential candidate rich in anthocyanins, and can be used as a good source for producing red dye for food [13, 14].

Extraction of natural dyes can be carried out by various methods such as the Soxhlet method, conventional solvent extraction, microwave-assisted [15, 16] and assisted ultrasonic or sonication [17]. Sonication is an extraction method that uses wave energy so that the formation of small bubbles is caused by the transmission of ultrasonic waves to diffuse solvents into the cell walls. The advantages of the sonication process are higher yield and faster extraction time [17].

The purpose of this study was to obtain anthocyanin pigments at the optimum extraction conditions from roselle flowers. The optimization of the method was carried out by analyzing the effect of ultrasonic wave frequency, process temperature, extraction time, and mass ratio of roselle flowers to the volume of distilled water on the anthocyanin concentration. The qualitative and quantitative compositions of the anthocyanin extract of rosella flowers were analyzed by UV-Vis spectrophotometer.

2. Material and methods

2.1. Materials
In this work rosella flower was obtained from Larise Pharmacy in Malang city. The chemicals used HNO₃, 96% ethanol, KI, I₂, and ethylene glycol for this research were obtained from PT. Brataco Chemicals (Surabaya branch).

2.2. Experimental apparatus
The equipment in this research includes: glassware, and sonicator bath (Mosinix USA brand) used for anthocyanin extraction from rosella flowers. Analytical equipment like XRD (XPert Pro diffractometer) was used to determine crystallinity of sample. Sample was radiated with Cu Kα (k = 1,5418 Å) for every q = 0,020. FTIR sample spectrum was obtained from Shimadzu FTIR-8400s within a range 400 - 4000 cm⁻¹ and prepared as KBr pellet. Diffusion reflectance spectrum UV-Vis (UV-Vis DRS) in range of wavelength 250 - 800 nm by used spectrophotometer (JASCO, V-760 Diffuse Reflectance Model).

2.3. Experimental Procedure
Rosella flower samples or materials are crushed using a blender. After that, 0.5 g of rosella flowers were weighed, and added with 50 mL of distilled water. The extraction process was carried out in a temperature range of 30 to 80 °C for 1-5 minutes with a sonicator (Mosinix, USA) at a frequency of 24-40 Hz bath type. and a variety of solvents with a ratio of rosella and distilled water from 1:25 to 1:125. The total anthocyanin content (TAC) was determined by the pH-differential method [18]. Hence the anthocyanin extract obtained was measured by a UV-Vis spectrophotometer. The results of the extraction process will then be analysed qualitatively using a UV-Vis spectrophotometer and later quantitative testing will be carried out using the equation:

$$Total \ anthocyanin \ (ppm) = \frac{(A \times MW \times FP \times 1000)}{(\varepsilon \times I)} \ (1)$$

Explanation:
A : Absorbance
MW : molecular weight
FP : dilution factor
ε : coefficient of extinction
I : cuvette width
3. Results and discussion

3.1 FTIR Absorption spectra of anthocyanin

At the beginning of the study, the rosella used was prepared by crushing it first using a blender to have a larger surface area and causing more contact between the sample and the solvent so that more extraction results were obtained [13]. Then the extraction of anthocyanin pigments from rosella flowers was carried out by sonication method on various variables, namely wave frequency, process temperature, extraction time, and the mass-volume ratio of rosella flowers and aquadest solvent. Aquadest solvent is used because rosella contains glycosides from anthocyanins which are polar so that polar solvents are also used to attract anthocyanins in rosella. Sonication is an extraction method that uses wave energy so that the formation of small bubbles is caused by the transmission of ultrasonic waves to diffuse solvents into the cell walls. [14]

![Figure 1. Anthocyanin FTIR Spectra Image](image)

Anthocyanin FTIR Spectrum Image is presented in Figure 1. Figure 1 shows the FTIR spectrum of the spectral range in the wave band 4000 - 400 cm\(^{-1}\), which indicates the presence of absorption at different wave numbers. There is a wide absorption with a strong intensity which indicates the presence of an OH group at wave number 3446 cm\(^{-1}\). Spectrum results also show a sharp absorption with moderate intensity at the wave number 1631 cm\(^{-1}\) there is a sharp absorption which is thought to be the absorption of the C = O double bond. Overall, these functional groups correspond to the functional groups contained in the anthocyanin basic framework [18].

3.2 UV-Vis Absorption spectra of anthocyanin
The absorption spectra of anthocyanin extracts obtained from various variables can be seen in Fig. 2 (a-d). Based on the UV-Vis spectrophotometer test, it is known that the wavelength obtained is 516-517 nm. This is appropriate and indicates the presence of anthocyanin compounds in the extraction results, where the maximum absorption limit of anthocyanin pigments is 505-535 nm [5].

**Figure 2.** The absorption spectra of anthocyanin extracts

**Figure 3.** Results of extraction optimization using variables (a) frequency (b) time (c) temperature and (d) solvent volume
In the experiments that have been carried out, it was found that the optimal extraction as shown in Figure 3 (a-d) will use a frequency of 60% or the equivalent of 24 kHz, if using a high frequency such as 100% it can damage the pigments in the cell so that the concentration can decrease. In addition, the optimal extraction uses a temperature of 40 °C, if the temperature is too high it will damage temperature sensitive pigments while low temperatures do not have sufficient energy. At high temperatures it will damage delphinidin 3-O-sambubiose and cyanidin 3-O-sambubiose which is an anthocyanin group to become gallic and protective acids, while those that are more easily degraded are delphinidin 3-O-sambubiose compounds compared to cyanidine 3-O-sambubiose [19]. For the optimal time done in 5 minutes, so the longer the time the concentration will also increase. Then for the ratio used to get optimal results using a ratio of 1:25 it will get the optimal extraction and have a high anthocyanin concentration.

4. Conclusions
Anthocyanin extraction from rosella flowers can be obtained by the ultrasonic assisted method in a short time. Based on the FTIR spectrum of the spectral range in the wave band 4000 - 400 cm⁻¹, it shows that there is a functional group that corresponds to the functional group contained in the anthocyanin basic framework. Meanwhile, based on uv-vis spectrophotometer analysis, anthocyanin extract absorption spectra were obtained with a peak at a wavelength of 516-517 nm, which indicates the presence of anthocyanin compounds. The use of a frequency of 60% or the equivalent of 24 kHz is very good, if using a high frequency such as 100% it can damage the pigments in the cell so that the concentration can decrease. If extraction using high temperatures will damage pigments that are sensitive to temperature while low temperatures do not have enough energy. The longer the time, the concentration will also increase. Then for a smaller ratio (more solvent) the yield will decrease. The optimal operating conditions for the process of extracting roselle as a source of anthocyanin are at 40 °C, 24 kHz frequency, 5 minutes time, and 1:25 ratio.

5. References
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