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Level, color intensity, and antioxidant status of crude anthocyanin of Indonesian agricultural commodities

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Abstract

Anthocyanin is an organic chemical compound that causes red, purple, blue, and black colors in plants. Anthocyanin is usually used as a natural coloring agent that is safe to be consumed, used as natural color-based indicators, and used as an antioxidant to prevent free radicals that can prevent aging, cancer, and degenerative diseases. Anthocyanins can be found in various plants, such as purple sweet potatoes, purple cabbage, beetroot, mangosteen, roses, as well as rosella. Each commodity has different types and characteristics of anthocyanins. Investigation was performed to get more insight the level, colour intensity, and antioxidant status of crude anthocyanin which is isolated from agricultural commodities in Indonesia. The result showed the highest yield of simplicial powder and crude extract were purple sweet potato (45.80%) and beetroot (38.13%), while the highest anthocyanin level (288.38 mg/L), color intensity (5.6) and the best antioxidants activity (51.43 ppm) were occupied by rosella. Thus, rosella is the best anthocyanin sources among the Indonesian agricultural commodities based on the level, colour intensity, and antioxidant status point of view.

Keywords: anthocyanin, antioxidant activity, colour intensity, crude extract

Short title: Crude Anthocyanin from Indonesian Agricultural Commoditie

Introduction

Anthocyanin is an organic chemical compound that dissolves in polar solvents and gives red, orange, purple, and blue to black colors (Widjonarko et al., 2014). Anthocyanins are often utilized as natural dyes in food products. The colour appearance of food products is essential because it affects consumer perceptions and preferences. However, the colors added to food products are not always safe to consume. Manufacturers often use dangerous synthetic dyes such as rhodamine B (Red No. 3), which contain heavy metals. This indicates the need of natural dyes that are safe for consumption and one of them is anthocyanin, a part of the natural color group with an INS (International Numbering System) of 163 and an ADI (Acceptable Daily Intake) of 0-2.5 mg/kg body weight. This means that anthocyanin can be consumed daily according to the maximum limit without causing adverse effects on health.

Besides the function of being a natural pigment, anthocyanin also has several biological effects. Anthocyanin is an antioxidant compound that can prevent degenerative diseases. The mechanism of anthocyanin as an antioxidant is to quench the reactive radical by transferring single electrons and the abstraction of the hydrogen atoms from its phenolic groups (Khoo et al., 2017). Anthocyanin is also observed to be more active than common antioxidants such as ascorbate. Overall, there is evidence that anthocyanin has the potency to be developed as a functional food due to its benefits for health and well-being.

The vacuole of some plant's organs, such as crown of flower, leaves, fruit, seeds, and tubers are anthocyanin sources wherein Indonesia is one of the countries in the world that produces agricultural commodities as the potential source of anthocyanin. These commodities are purple sweet potato, beetroot, red rose, rosella, purple cabbage, and red spinach. Each commodity has a different type of anthocyanin. Generally, anthocyanins found in plants are pelargonidin, cyanidin, delphinidin, peonidin, petunidin, malvidin, and anthocyanidin glycosides (Khan et al., 2021). The type of anthocyanin contained in purple sweet potato is peonidin and cyanidin [38], while in red spinach, cyanidin is found (Oladzadabbasabadi et al., 2022). Red roses contain cyanidin and pelargonidin (Montilla et al., 2010), while rosella flowers contain cyanidin-3-glucoside (Oladzadabbasabadi et al., 2022).

Each commodity's different types of anthocyanins will provide different physical and chemical characteristics (Oladzadabbasabadi et al., 2022). Therefore, studies on the characterization of anthocyanins produced from various plant commodities need to be carried out before they are applied in agro-industrial fields, especially in the food sector. The characteristics of

anthocyanin extracts can be evaluated from various parameters such as anthocyanin levels, antioxidant activity, and redness intensity.

The high anthocyanin level is expected to be related to the redness intensity and the antioxidant activity. Anthocyanins contain phenolic hydroxyls acting as free radical reducers responsible for antioxidant activity (Shavisi & Shahbazi, 2022). It is necessary to confirm the correlation between anthocyanin levels, the intensity of the red color, and the antioxidant status. Therefore, the investigation was performed to get more insight into the level, colour intensity, and antioxidant status of crude anthocyanin isolated from agricultural commodities in Indonesia. Thus, rosella is the best anthocyanin source among Indonesian agricultural commodities based on the level, color intensity, and antioxidant status point of view.

Materials and Methods

Materials

The materials used in this study were red rose, red rosella, beetroot, purple sweet potato, red spinach, red cabbage, red dragon fruit, and mangosteen (Table 1), 96% ethanol, 3% citric acid, methanol, HCl 1%, 1,1-diphenyl-2-picrilhydrazyl (DPPH), buffer solution of HCl-KCl with pH 1 and pH 4.5 respectively. All chemicals utilized in this research are analytical grade.

Table 1. Various and Physical Appearance of Agricultural Commodities as Anthocyanin Sources

Commodities	Harvest Location	Appearance	
Red rose (<i>Rosa hybrida</i> L)	Batu, East Java	Red in whole petals with a little bit dark color on the edge of the petals.	
Red rosella (<i>Hibiscus sabdariffa</i>)	Yogyakarta, DIY	Red in whole petals with a little bit of white colour on the center	
Purple sweet potato (<i>Ipomoea batatas</i>)	Bandungan, Central Java	Light purple with a solid texture.	
Beetroot (<i>Beta vulgaris</i>)	Bandungan, Central Java	Dark red in whole fruit. The texture is watery.	
Red Cabbage (<i>Brassica oleracea</i> var. <i>capitata</i> f. <i>rubra</i>)	Bandungan, Central Java	Purple colour with a little bit of white colour in the middle of the cabbage	

Red Spinach (Amaranthus tricolor-var Blitum rubrum)	Bandungan, Central Java	Purple colour without any green part of the leaf	
Mangosteen fruit (Garcinia mangostana)	Semarang, Central Java	Dark purple, the fruit is ready to consume. The sap has been lost, and the fruit's content is easily released.	
Red dragon fruit (Hylocereus polyrhizus)	Semarang, Central Java	Red color, the fruit is ready to consume with a sweet taste. The content of the fruit is easily released.	

Methods

Sample Preparation

First, samples were cleaned using water to remove all impurities, dried by a cabinet dryer (50°C) to get the dry matter weight (moisture content less than 10%), and finally powdered.

Extraction of Anthocyanin

Anthocyanins extraction was performed through the solvent extraction and maceration method based on Ćujić et al., (2016) and Silva et al., (2017) with slight modifications. The samples were cleaned, sliced, and dried in a cabinet dryer (50°C) until the water content was less than 10%. The dried sample was then mashed with a blender and macerated for 3 x 24 hours in a 96% v/v ethanol solvent mixed with 3% citric acid (w/v). Samples were macerated in a closed container covered with aluminium foil, placed in a dark place at room temperature, and then separated residue from the filtrate by a paper filter. Finally, the filtrate was evaporated inside the cabinet dryer (50 °C for 8 hours).

Yield Rate Determination

The yield rate is the percentage of weight between the weight of the extract produced and the weight of the simplicial used as raw material. A high yield value indicates that the resulting extract is getting bigger. The yield percentage was determined by dividing the total weight of pigment concentrate (g) by the weight of the powdered sample (g).

Anthocyanin Level Measurement

Anthocyanin levels were measured using the pH differential method using UV-Vis spectrophotometry, as compared to the previous study (Molaeafard et al., 2021). The anthocyanin extract sample of 1 g was diluted with 1% HCl in methanol to extract the anthocyanins in the sample. The sample was allowed to stand for 16 hours, and the solution

was centrifuged. The result of the clear filtrate was taken at 1 ml and put into 2 test tubes. The first test tube of the extracted sample was added with 9 ml of HCl-KCl buffer solution pH 1, while the second test tube was added with 9 ml of acetate buffer solution pH 4.5, then each test tube was vortexed. The absorbance of the two treatments was measured at a wavelength of 250 nm to 700 nm to obtain the results of anthocyanin levels. The determination of total anthocyanin levels in the sample is calculated using the formula conducted in the previous study (Netravati et al., 2022).

Antioxidant activity Determination

Some indicated concentration samples were stored in a test tube, and then 1 ml of DPPH solution (1,1-diphenyl-2-picrylhydrazil) 200 μ M was added into each test tube. Blanko was prepared by mixing 4 ml ethanol and 1 ml DPPH solution. Samples and blank were incubated in the dark room (30 minutes), and the absorbance wavelength was adjusted to 517 nm. The inhibition percentage was calculated by the formula: $\text{Inhibition (\%)} = \frac{\text{OD Blanko} - \text{OD Sample}}{\text{OD Blanko}} \times 100\%$. The inhibition percentage and sample concentration (ppm) are plotted in the standard curve and figured out based on the Vitamin C sample. The x value is calculated by changing the y value to 50 (IC_{50}) is the antioxidant concentration (ppm).

Colour intensity measurement

The color intensity analysis was carried out using the CR-400 Chromameter tool. The sample is placed into a white plate, and the measured numbers will displayed on the screen. The marks of lightness (L^*), redness (a^*) and yellowness (b^*) will be shown and recorded on the screen.

Data analysis

The data obtained from the test results were analyzed descriptively to compare anthocyanin's characteristic between commodities. The data of anthocyanins levels, antioxidant activity, and colour intensity were analysed use Pearson correlation method to determine the relation between the parameters with the significance level of 0.05. The direction of correlation can be seen from the sign of the correlation coefficient as described in the previous study (Chen et al., 2021).

Results and Discussion

Yield average

Yield is the ratio of the sample's weight before going through some processes and the sample's weight after the process. In this study, the yield rate was divided into two; (1) the yield

of the drying process to make simplicial powder and (2) the yield of the extraction process (Table 2).

Table 2. Yield rate of simplisia powder and anthocyanin crude extract from various agricultural commodities

Sample	Yield (%)	
	Simplisia Powder	Crude Extract
Red Rose	19.95±5.65	27.08±5.98
Red Rosella	20.11±7.77	32.04±8.23
Beetroot	10.08±3.21	38.13±7.26
Purple Sweet Potato	45.80±8.21	22.92±6.22
Red Cabbage	8.80±0.75	28.31±6.16
Red Spinach	14.44±3.21	24.33±5.89
Mangosteen Fruit	27.64±7.21	32.16±6.21
Red Dragon Fruit	34.08±6.82	18.65±3.34

The yield test results showed that the highest drying yield was purple sweet potato, 45.80%, followed by red dragon fruit, mangosteen, rosella, roses, red spinach, beetroot, and red cabbage. The lowest drying yield was red cabbage, which was 8.80%. The amount of water content in each sample affects the drying yield rate. According to Wang (Wang et al., 2020), the water content of purple sweet potatoes ranged from 68.03% to 76.70%, beetroot 87.58%, red spinach 86.85%, and purple cabbage 91.98% (Wang et al., 2020). The purple sweet potato has a lower water content than other samples. In contrast, purple cabbage has the highest water content. Therefore, during the drying process, the purple sweet potato did not lose weight as much as the purple cabbage.

The result shows that the highest extract yield value was beetroot samples (38.13%). It shows that the extraction process of beetroot was carried out effectively. Differences in extract yields can be caused by differences in where each commodity grows and the number of solids or chemical compounds contained in the extract. However, extract yield is not only influenced by the number of anthocyanins contained. According to Zhang (USDA, 2014), the extracted impurities also increased the yield value. These impurity compounds can consist of sugars, organic acids, fats, proteins, minerals, and other phenolic compounds besides anthocyanins, contributing to the increase in yield value. The high extract yield in beetroot can also be caused by the ethanol solvent, which has not completely evaporated during the evaporation process, so the remaining ethanol also contributes to the weight of the extract.

Anthocyanin levels

Anthocyanin levels are determined through the crude anthocyanin yielded from various agricultural commodities such as red rose, red rosella, beetroot, sweet potato, red cabbage, red spinach, mangosteen fruit, and red dragon fruit amount of anthocyanin extracted from the samples (Figure 1).

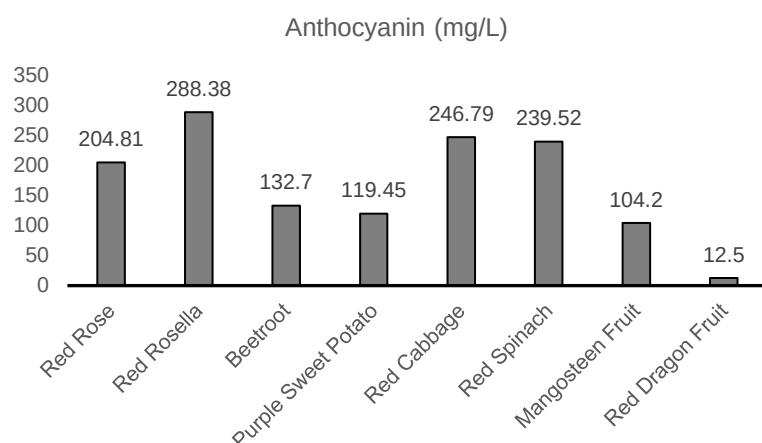


Figure 1. Anthocyanin levels (mg/dL) which were detected from crude extract of various agricultural commodities

The highest anthocyanin levels in this research were produced by a rosella flower sample of 288.38 mg/L, followed by red cabbage, red spinach, roses, beetroot, purple sweet potato, mangosteen fruit, and red dragon fruit. The high number of anthocyanins in rosella is also affected by the type of anthocyanins contained. Rosella flowers contain cyanidin-3-glucoside, cyanidin-3-sambubioside (which has two OH groups in ring B), delphinidin-3-glucoside, and delphinidin-3-sambubioside (which has three groups of OH in ring B). Therefore, it can stabilize anthocyanins in rosella flowers. In addition, the organic acid content in rosella also increased total anthocyanin levels. Naturally, rosella contains dominant organic acids, such as ascorbic acid (18.85 %), citric acid (13.23%), malic acid (20.45%), and other organic acids (Sindi et al., 2014). The acidic conditions in rosella flowers make anthocyanins stable in the form of flavylium or oxonium cations, which are red during the extraction process (Widjonarko et al., 2014).

The data showed that each sample had different anthocyanin levels. The differences are due to differences in the composition of each commodity. Each commodity has a different ability to synthesize anthocyanins due to genetic diversity in expressing the genetic code to produce an active compound (Sukkhaeng et al., 2018). Differences in anthocyanin levels can also be affected by growing environmental conditions such as climate, altitude of the place, and

soil pH (Li et al., 2018). Genetic and environmental factors of growing a plant will impact the type and structure of anthocyanins. In addition, differences in anthocyanin levels can be caused by differences in harvest age related to size, material color, and maturity level.

The anthocyanin levels are also affected by the extraction method. This research used ethanol with citric acid as the solvent to optimize the extraction. Based on Yuniati et al, (2024), alcohol such as ethanol is a better solvent of anthocyanin's maceration than aquadest solvents. Ethanol is a suitable solvent for anthocyanin extraction because it has a lower boiling point than aquadest, so the evaporation process is faster than aquadest.

Acidity can cause cell membranes to denature, dissolve, and stabilize the anthocyanins compounds (Ouerghemmi et al., 2016). Using acid stabilizes anthocyanins in the form of red flavilium cations at low pH. However, solvents acidified using strong acids such as hydrochloric acid can hydrolyze anthocyanins. anthocyanin extraction using ethanol acidified with citric acid (0.01%) rather than hydrochloric acid is preferred for use in food because citric acid is a food-grade acid, colourless, non-corrosive compared to hydrochloric acid (Garcia-Mendoza et al., 2017).

Antioxidant activity of crude anthocyanin

Antioxidant power can be determined based on the IC₅₀ value. IC₅₀ value indicates the concentration of compound that is required to scavenge DPPH radicals by 50%. The smaller the IC₅₀, the stronger antioxidant activity (Abou-Arab et al., 2011). IC₅₀ less than 50 ppm is categorized as a very strong antioxidant, 50-100 ppm as the strong antioxidant, 100-250 ppm as the intermediate antioxidant, 250-500 ppm as the poor antioxidant, and IC₅₀ worth more than 500 ppm is rated as the inactive antioxidant (Erkan, 2012). Based on that category, dragon fruit is classified as weak antioxidants. Purple sweet potatoes, beetroot, red spinach, red cabbage, mangosteen, and roses are classified as intermediate antioxidants. Meanwhile, rosella is classified as a high-power antioxidant. Various of antioxidant status is affected by several factors, such as the sample capacity to release hydrogen atoms to DPPH free radicals, the compound chemical structure, and the amount of hydroxyl groups (Jun et al., 2003).

The result of this research show that the sample with the best antioxidant activity was rosella extract which had IC₅₀ of 51.43 ppm and classified as a powerful antioxidant. Previous study reported that rosella has a potent antioxidant with IC₅₀ value of 129-62 ppm (Yeo & Shahidi, 2019). Variation of IC₅₀ value in the similar commodity likely was caused by some factors such as the differences of the solvent, extraction method, and maturity level of the rosella. This research used 96% ethanol and 3% citric acid as the solvent, meanwhile previous study used

aquadest as the solvent. It shows that, ethanol and citric acid as the solvent can extract rosella's antioxidant better than aquadest.

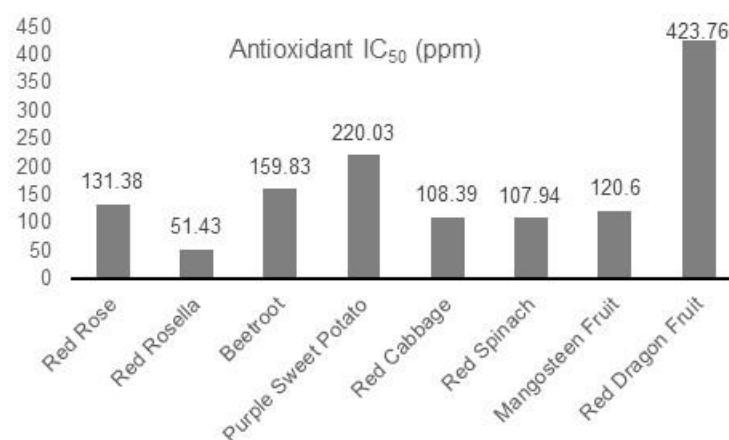


Figure 2. Antioxidant activity of crude anthocyanin extracted from various agricultural commodities. Data of antioxidant activity is expressed as Inhibitory Concentration Fifty (IC₅₀) value.

The high anthocyanin content has a positive correlation to the antioxidant potency of rosella (Djaeni, 2017). Anthocyanins have an antioxidant activity 2-6 times higher than other antioxidant compounds, such as ascorbic acid and glutathione (Tian et al., 2019). In addition, other components of rosella also affected the antioxidant activity. Another factor influences the antioxidant activity of rosella is the content of the organic compounds such as vitamin C, hydroxybenzoic acids, hydroxycinnamic acids, and flavanols (Christian & Jackson, 2009; Prior et al., 1998).

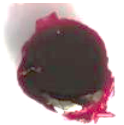
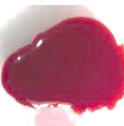
Color intensity

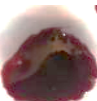
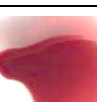
The colour intensity of crude anthocyanin was measured using a chromameter tool to determine the redness intensity (Table 5). All samples tested showed different values of L*, a*, and b*. Differences in the crude extract colour depend on the source varieties and extraction conditions such as solvent type, concentration, extraction time, and temperature (Singh et al., 2021). In addition, the color of each commodity's extract depends on the concentration and type of anthocyanins contained because the colour shown by each fruit, vegetable, or flower is not only produced by a single pigment but a combination of several pigments (Jung et al., 2013). The anthocyanin contained in the sample impacted to the colour density of the crude extract because the more pigment combinations in the sample, the more intense the colour will be. The

colour density of the crude extract was expressed as lightness (L^*) redness (a^*), and yellowness (b^*).

The test results showed that the sample with the highest a^* (redness) value was rosella, which had a^* value of 5.6, an L^* value of 19.96, and b^* value of 2.58. Then, followed by rosella, purple cabbage, rose, red spinach, red dragon fruit, beetroot, purple sweet potato, and mangosteen fruit. The combination of pigments causes rosella samples to have a high redness value. Rosella flowers contain anthocyanins such as cyanidin and delphinidin, which responsible for the dark red, purplish red, and bluish red of the rosella extract (Jung et al., 2013). Cyanidin and delphinidin are more stable in maintaining their colour in acidic conditions (Widjonarko et al., 2014).

Table 5. Comparison of lightness (L^*), redness (a^*), and yellowness (b^*) of crude anthocyanin extracted from various agricultural commodities

Sample	Lightness (L)	Redness (a)	Yellowness (b)	Description	Picture
Red Rose	23.73 ± 0.183	04.87 ± 0.106	3.44 ± 0.06	The extract was very thick, dark red, makes marks when washed.	
Red Rosella	19.96 ± 0.014	05.60 ± 0.283	2.58 ± 0.01	The extract was thick, dark red, did not make marks when washed.	
Beetroot	22.01 ± 0.007	04.38 ± 0.092	2.69 ± 0.02	The extract was thick and dark red, makes marks when washed.	
Purple Sweet Potato	21.10 ± 0.014	03.55 ± 0.071	4.19 ± 0.04	The extract was slightly thick and red, did not make marks when washed.	

Red Cabbage	19.90 ± 0.014	05.31 ± 0.014	2.40 ± 0.01	The extract was thick and dark red, makes marks when washed.	
Red Spinach	31.54 ± 1.407	03.65 ± 0.092	2.85 ± 0.08	The extract was thick and dark red, makes marks when washed.	
Mangosteen	22.57 ± 0.014	03.00 ± 0.141	5.15 ± 0.01	The extract was slightly thick, red-brown, did not make marks when washed.	
Red Dragon Fruit	19.73 ± 0.176	04.49 ± 1.138	3.31 ± 0.10	The extract was slightly thick, yellow-brown, did not make marks when washed.	

In addition, the high colour intensity in the rosella extract is thought to occur due to the high organic acid content. Naturally, rosella flowers contain dominant organic acids, such as ascorbic acid, citric acid, malic acid, and other organic acids (Sindi et al., 2014). The organic acid content in rosella flowers will keep flavylum cation with the result anthocyanins become more stable which is manifested by stronger colour intensity.

Correlation of anthocyanins, antioxidant activity, and redness intensity

The anthocyanin levels and the IC₅₀ value showed a strong significant correlation (Table 6). A negative correlation coefficient indicates that the two parameters have an inverse correlation which is the higher the anthocyanin level, the lower the IC₅₀ value. The IC₅₀ value indicates the sample concentration required to scavenge 50% free radicals. Therefore, the smaller the IC₅₀ value, the better the antioxidant activity. The redness intensity and the anthocyanin levels have a positive strong correlation (Table 6), which is the higher anthocyanin

level, the stronger the redness intensity of the extract (Bursać Kovačević et al., 2015). The higher the anthocyanin content in crude extract, the redder the sample (Sommer & Cohen, 2018). High concentrations of anthocyanin extract correlated with its color. At high concentrations, the anthocyanin extract is red, while at low concentrations, the anthocyanin extract is bluish.

Table 6. Correlation of anthocyanins, antioxidant activity, and redness intensity

Parameters		Antioxidant Activity (IC ₅₀)	Redness Intensity (*a)
Anthocyanins	Correlation	-0.858	0.946
	Coefficient		
	Significance	0.006	0.000

4. Conclusions

Several conclusions were obtained based on comparing the level, color intensity, and antioxidant status of crude anthocyanins from various agricultural commodities in Indonesia. Purple sweet potatoes produced the highest yield of simplicia powder, but beets produced the highest yield of crude anthocyanin oil extract. Among the eight commodities tested, rosella had the highest antioxidant activity, red color intensity, and antioxidant levels. These results can be a reference for recommendations on good sources of anthocyanins for Indonesian source-based functional food and nutraceutical products.

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