Effect of blanching, acid type, and temperature on the extraction of anthocyanin from *Tibouchina semidecandra* flower

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**Abstract**

Extraction is the most common way to obtain anthocyanins from their natural matrices. During the extraction, the anthocyanin may degrade due to the heat and acidic solvent. The objectives of this research were to determine the best acidic aqueous solution and temperature to extract anthocyanin from *Tibouchina semidecandra* flower and to decide if the blanching process has a significant effect on the extracted anthocyanin. The *T.* *semidecandra* petals were separated into two groups: steam blanched for 6 mins and without blanching. Both were dried for 24 hrs at 45°C and powdered. The extract was prepared by macerating the powdered petal into pH 3 aqueous solutions made of three different acids (citric, malic, and hydrochloric acid, respectively) in a water bath shaker at various temperatures (30, 45, 60, and 75°C) for an hour. The extracts were then filtered, centrifuged, and analysed for their colour intensity, browning index, monomeric anthocyanin, polymeric anthocyanin, and total phenolic content. The best method to obtain the highest colour intensity, monomeric anthocyanin, total phenolic content, and lowest browning index was using steam-blanched dried petal, citric or malic acid as solvent and extraction temperature at 60°C. The total monomeric anthocyanin and phenolic content of the extract was 3.63±0.35 mg/g dried petal and 74.47±11.47 mg/g dried petal, respectively.

1. **Introduction**

The potential adverse health effect of a long-period consumption of artificial colourant in food to the human body increased the demand for natural pigment. Anthocyanins are amongst the most potent natural food colourants that also provide health benefits to the human body. These pigments are water-soluble and exhibit a wide range of colours from red to blue, depending on their chemical structure and pH.

The stability of anthocyanins is affected by several factors, particularly pH and heat. The colour may also be interfered by the brown colour due to the activity of the polyphenol oxidase enzyme (Patras et al., 2010). A mild heating treatment was reported to inactivate the enzyme. However, the heat exposure during blanching could probably decrease the anthocyanin content (Patras et al., 2010).

*Lasiandra* (*Tibouchina semidecandra* L.) is an ornamental shrub producing dark purple flowers that are also used traditionally in medicine and food (Janna and Khairul, 2007). The flower was reported to have a monoacylated anthocyanin identified as malvidin-3- (p-coumarylglucoside)-5-glucoside (Lowry, 1976). Anthocyanins containing acyl groups are more stable than those with no acyl group (Marpaung et al., 2015). Janna and Khairul (2007) reported that the anthocyanin from *T. semidecandra* was relatively stable at pH 3 or below. Meanwhile, Sun et al. (2011) described that the anthocyanin degradation at a high acidic condition was initiated by the hydrolysis of the anthocyanin to its aglycon. Hypothetically, the strong acid would cause more severe hydrolysis than the weak acid.

Referring to the background, we considered that the application of blanching, different acid types and elevated temperature was necessary to be studied to obtain the best extraction parameter of anthocyanin from *T. semidecandra*. The colour intensity, browning index, total monomeric, percentage of polymeric anthocyanin, and total phenolic content were measured to the quality of the anthocyanin extracts.
2. Materials and methods

2.1 Material

*Tibouchina semidecandra* petals (TS) were gathered from Taman Nasional Gunung Salak-Halimun, West Java, Indonesia. The petals were divided into two, one underwent the steam blanching process for 6 minutes (Marpaung et al., 2013) and the rest was left without blanching. Both blanched and non-blanched petals were dried in an oven (Daihan Wiseven, Korea) for about 24 hrs at 45°C. The dried petals are powdered and kept in a freezer until used. Hydrochloric acid, potassium chloride, sodium carbonate, Folin-Ciocalteau reagent, gallic acid, were obtained from Merck® (Germany). The reagents were analytical grade. Citric acid and malic acid were food-grade (Brataco, Indonesia).

2.2 Extraction

The extraction was done by macerating the powdered petals in three types of acidified distilled water pH 2 (citric acid, malic acid, and hydrochloric acid) at various temperatures (30, 45 60, and 75°C) for an hour with continuous shaking and without the presence of light. The volume of solvents was 40 mL per 1 g powdered petal. The extract was filtered using filter clothe, then centrifuged at 7000 rpm at 25°C for 5 mins. The colour quality, monomeric anthocyanin content, polymeric anthocyanin, and total phenolic content of every treatment were determined.

2.3 Colour quality

The colour quality of an anthocyanin source extract was commonly measured by its colour intensity (CI) and browning index (BI) (Marpaung et al., 2019). The wavelength giving the highest absorbance (λ_max) at visible light region of the extract was determined by a UV-Vis spectrophotometer (Genesys 10uv Thermo Electron Corporation, USA). The CI of the extract calculated as follow:

\[ CI = (\lambda_{\text{max}} - \lambda_{700}) \times DF \]  

Where \( \lambda_{\text{max}} \) and \( \lambda_{700} \) is the absorbance at \( \lambda_{\text{max}} \) and 700 nm respectively, and DF is the dilution factor.

Meanwhile, the BI determined as follow:

\[ BI = (A_{420} - A_{700}) / (A_{\lambda_{\text{max}}} - A_{700}) \]  

Where \( A_{\lambda_{\text{max}}} \) is the absorbance at \( \lambda_{\text{max}} \), \( A_{420} \) is the absorbance at 420 nm, and \( A_{700} \) is the absorbance at 700 nm, and DF is a dilution factor.

2.5 Total monomeric anthocyanin

The total monomeric anthocyanin (AM) was determined by the pH differential method (Marpaung et al., 2013) and calculated as malvidin-3-glucoside. The principle of the method was by measuring the highest absorbance at the visible light region (absorbance at \( \lambda_{\text{max}} \)) of the extract at pH 1 and 4.5 using a UV-Vis spectrophotometer. At pH 1, the only species that existed in an anthocyanin was red flavylum cation (AH^+). Meanwhile, at pH 4.5 the predominant species was colourless hemiketal (B). The colour appeared at pH 4.5 representing the degraded anthocyanins in the polymeric form that are resistant to colour change with a change in pH. Hence, the difference between the absorbance at pH 1 and 4.5 represent the intensity of the monomeric anthocyanin.

\[ A = (A_{\lambda_{\text{max}}} - A_{700})_{\text{pH } 1.0} - (A_{\lambda_{\text{max}}} - A_{700})_{\text{pH } 4.5} \]  

By using the following formula the total monomeric anthocyanin in the extract (mg/L) could be determined.

\[ AM = (A \times MW \times DF \times 1000) / (\varepsilon \times l) \]  

Where MW is the molecular weight of malvidin-3-glucoside (493.2 g/mol), DF is a dilution factor, \( \varepsilon \) is the molar absorptivity of malvidin-3-glucoside (28000), and \( l \) is the cuvette width (1 cm).

2.4 Polymeric anthocyanin

The percentage of polymeric anthocyanin (AP) was measured by treating the sample with sodium metabisulphite (Brownmiller et al., 2008). A polymeric anthocyanin is resistant to bleaching by bisulphite, on the other hand, the monomeric anthocyanins will be bleached and become colourless.

Each 2.8 mL of sample was put into two cuvettes. Then 0.2 mL of bisulphite was added into one cuvette, and as the control sample, 0.2 mL of distilled water was added into another cuvette. Then the mixtures were equilibrated for 15 mins. The absorbance of each sample was measured using a spectrophotometer at 420 nm, \( \lambda_{\text{max}} \), and 700 nm. The colour density of the control sample was calculated as follow:

\[ \text{Colour density} = [(A_{420} - A_{700}) + (A_{\lambda_{\text{max}}} - A_{700})] \times(5) \]

\[ \text{Dilution factor} \]

The polymeric anthocyanin content was calculated as follow:

\[ \text{Polymeric colour} = [(A_{420} - A_{700}) + (A_{\lambda_{\text{max}}} - A_{700})] \times(6) \]

\[ \text{Dilution factor} \]

The percentage of polymeric colour is calculated as follow:

\[ \text{AP} = \text{(Polymeric colour)} / \text{(Colour density)} \times 100 \]  

2.5 Total phenolic content

The Folin-Ciocalteau method was used to measure the total phenolic content (TP) of the extracts and stated as the gallic acid equivalent (GAE) (Marpaung et al., 2013). A 0.2 mL extract was added to 0.8 mL of 20%
Na$_2$CO$_3$ and 1 mL Folin-Ciocalteau reagent. The mixture was left for one hour, then the absorbance was measured at 765 nm. The standard curve was developed by the reaction of 0.2 mL gallic acid at several levels of concentration with 0.8 mL of 20% Na$_2$CO$_3$ and 1 mL Folin-Ciocalteau reagent. The TP was calculated as follow.

$$\text{TP (mg/L GAE)} = \frac{\text{Abs}}{m}$$  \hspace{1cm} (8)

Where Abs is the absorbance (A) and m is the slope of the gallic acid standard curve.

2.6 Statistical analysis

Statistical analyses involved in this experiment were 3 ways Analysis of Variance (ANOVA) (Design-Expert® version 7.0.0., Stat-Ease, Inc., Minneapolis, USA) and followed by Tukey HSD test for the posthoc analysis (OpenStat® version 11.9.08).

3. Results and discussion

Total monomeric anthocyanin of all TS extracts studied ranged from 26.77 to 103.13 mg per litre or equal to 1.07 to 4.13 mg/g dried petal. The colour intensity and browning index of TS extracts were 2.28 to 10.72 and 0.36 to 0.69, respectively. The total phenolic content of TS extracts was 810.21 to 2234.41 mg/L GAE or equal to 32.41 - 89.38 mg/g dried petal. The percentage of polymeric anthocyanin fraction in the TS extracts was 20.19 - 27.09%.

3.1 Effect of blanching of the petal prior to drying

The blanching process was applied to the flower before drying to avoid the brown colour development due to the activity of the polyphenol oxidase enzyme (Marpaung et al., 2013). On the other hand, the heat applied during blanching damage the cell wall of the flower to increase the yield of the anthocyanin extracted (Marpaung et al., 2013; Liu et al., 2015; Deylami et al., 2016; Mahmudatussa'adah et al., 2019). In short, blanching is often to be applied to increase the colour quality of an anthocyanin source extract.

As seen in Figure 1, the blanching increased the colour quality of TS extract by giving significant higher CI and lower BI (the p-value was 0.0006 and 0.0015, respectively). Browning index was calculated by dividing the absorbance at 420 nm ($A_{420}$) by the absorbance at $\lambda_{\text{max}}$ ($A_{\text{max}}$). The $A_{420}$ of blanched and non-blanched extracts was not significantly different. Therefore, the higher BI in non-blanched extracts was contributed only by their lower CI. Hence, the six minutes of steam blanching prior to drying improve the effectiveness of the extraction, but was not significant to avoid browning.

Figure 1. The effect of blanching process to the colour intensity (CI), monomeric and polymeric Anthocyanin (AM and AP, respectively), browning index (B)) and total phenolic content (PC) of the extract of $T$. semidecandra flower. Bars with the same notations in the same graph are not significantly different ($\alpha = 0.05$).

A wide variation of the duration of steam blanching to inactivate polyphenol oxidase has been reported. Li et al. (2019) reported that one-minute steam blanching is adequate to inactivate almost 100% of polyphenol oxidase in the Chrysanthemum indicum flower. Cevallos-Casals and Cisneros-Zevallos (2004) stated that 10 mins steam blanching is needed to ensure inactivation of polyphenol oxidase in Andean purple corn and red-fleshed sweet potato. Zhang et al. (2011) reported that the inactivation of polyphenol oxidase of Echinacea purpurea roots was achieved by steam blanching for 15 mins. Hence, further study to obtain the best time to avoid browning in TS flowers is needed.

Besides increasing the colour quality, blanching also increased the potentiality of TS extract as the bioactive compound source by yielding higher anthocyanin and phenolic content (the p-value was 0.0102, and 0.0467, respectively). Meanwhile, the blanched sample had a lower percentage of polymeric anthocyanin (p-value = 0.0003), which could be because of the increase in the colour intensity.

3.2 Effect of acid type

Figure 2 shows that weak acid (citric acid and malic acid) was better to extract the anthocyanin from $T$. semidecandra petal. A similar result for different anthocyanin source extracts like Berberis vulgaris, Solanum melongena, Brassica oleracea (Hosseini et al., 2016), Carissa carandas (Le et al., 2016) and Ficus carica L. (Meziant et al., 2018) was also recommended previously.

The CI of the anthocyanin extracted by hydrochloric acid (HA-extract) was significantly lower than the CI of anthocyanin extracted with citric and malic acid (CA-extract and MA extract, respectively). Hydrochloric acid is an effective solvent to extract anthocyanin (Hosseini et al., 2016). However, it also tends to degrade most organic
compounds (Hosseini et al., 2016). Therefore, the lower CI of HA-extract was probably because of the degradation of the anthocyanin. This probability is supported by the two other characteristics of HA extract: lower AM and lower TP content.

Marpaung et al. (2019) described that the anthocyanin degradation initiated by the hydration of the red colour species (flavylium cation) to colourless species (hemiketal), indicated by the decrease of colour intensity. In the extract extracted by a weak acid, the hydration might be blocked because of the citric acid that chelates anthocyanin and builds copigmentation that stabilise the colour (Hosseini et al., 2016). The work of malic acid to configure copigmentation with anthocyanin was also evident (Munawaroh et al., 2016). The next step of anthocyanin degradation is the tautomerization of colourless species to pale yellow species, known as chalcone. The degradation is indicated by the increase of BI. As shown in Figure 2, a significant increase of BI was observed in HA extract.

The colourless hemiketal and chalcone were anthocyanin and calculated in the total monomeric anthocyanin determination. Hence, the decrease in colour intensity was not always mean the decrease of total monomeric anthocyanin. However, this study showed that the AM in HA extract was significantly lower than the AM in CA and MA extract. This result indicated that during the extraction by hydrochloric acid apart of anthocyanin in TS degraded further to simple products like benzaldehyde and benzoic acid derivatives (Marpaung et al., 2017b). Because of the degradation, the total phenolic content in HA extracts also decreased.

3.3 Effect of temperature

Heat is the energy commonly applied to release bioactive compounds like anthocyanins from their natural matrices. However, heat is also a significant factor that may degrade anthocyanin. Therefore, the extraction of anthocyanin from a plant at a moderate temperature reported to be the best.

Wang et al. (2016) reported that the optimum parameters to extract anthocyanin from blueberries, and red pear peels are 50°C for an hour. Marpaung et al. (2013) reported that extraction at 60°C for 30 mins is optimum to extract the anthocyanins from Clitoria ternatea petal. With microwave-assisted extraction, the best condition to extract anthocyanins from blueberry powder is at 47°C for 7 minutes (Zheng et al., 2013). The use of higher temperature and longer time (70°C for 2 hours) are reported to extract anthocyanin from black rice bran (Kim et al., 2015).

The optimum extraction of the anthocyanin from the TS petal was also achieved by applying a moderate heat treatment. As seen in Figure 3, the highest CI, AM, and TP of TS extract were yielded through the extraction at 60 and 75°C for an hour. There was no significant difference in colour intensity and anthocyanin content caused by the two temperatures. Hence, 60°C was preferred because of the lower energy consumption.
3.4 Best extraction parameter

With the help of Design-Expert software, the best extraction condition to obtain the highest colour intensity, total monomeric anthocyanin and total phenolic content were determined. The chosen process was blanching prior to drying, the use of citric acid or malic acid as solvent and 60°C as the temperature of the extraction. The predicted quality of the extract is listed in Table 1.

The CI and BI of TS extract were relatively comparable to the CI and BI of the various plant extract like Clitoria ternatea, Melastoma malabathricum, Rhodomyrtus tomentosa and Bauhinia purpurea (Marpaung et al., 2015; Marpaung et al., 2017a). For the AM, TS extract showed a higher content of AM than the extract of eggplant (1.38 mg/g dried petal) (Dranca and Oroian, 2016), red cabbage (0.3 mg/g dried leaves) (Oroian et al., 2017). However, the AM of TS extract was lower than the AM of blueberry (8.09 mg/g dried fruit) (Darniadi et al., 2019). The percentage of polymeric anthocyanin fraction in the TS extract was relatively close to the percentage of polymeric anthocyanin of other anthocyanin source extract, like black carrot juice (15.66 to 22.32%) (Türkyılmaz et al., 2012) and strawberry pulps (22.61 - 29.25%) (Cao et al., 2011). The phenolic content of TS extracts was higher than the phenolic content of blueberry g (Darniadi et al., 2019), eggplant peel (Dranca and Oroian, 2016) and red cabbage (Oroian et al., 2017).

Table 1. Quality of the extract of blanched Tibouchina semidecandra petal powder extracted by citric acid at pH 2, 60°C for an hour

| Quality Parameter                  | Unit                   | Value (Mean±95% confidence interval) |
|------------------------------------|------------------------|--------------------------------------|
| Colour intensity                   | Absorbance Unit (AU)   | 7.70±0.73                            |
| Browning index                     |                        | 0.39±0.024                           |
| Total monomeric anthocyanin        | mg/L                   | 90.75±8.69                           |
| Polymeric anthocyanin              | mg/g dry petal         | 3.63±0.35                            |
| Total phenolic content             | mg/L                   | 1861.93±286.80                       |
|                                    | mg/g dry petal         | 74.47±11.47                          |

Conflict of interest

The authors declare no conflict of interest.

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