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Anthocyanin Extraction from Cockspur Coral (*Erythrina crista-galli*) Flowers Using a Microwave-Assisted Extraction (MAE) Method: Effect of Microwave Power and Feedstock-to-Solvent Ratio

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ABSTRACT

Anthocyanin is a water-soluble compound as a coloring agent that can give rise colors in plants such as Cockspur Coral flowers. Cockspur Coral flowers contain antioxidants in the form of polyphenol compounds. This study investigated the effect of extraction time and the use of microwave power on anthocyanin concentrations in the microwave-assisted extraction (MAE). The use of MAE was beneficial due to its short extraction time, low need of solvent, and high yields of the target compounds. A 10 g of Cockspur Coral flowers was mixed with 4% of solvent containing citric acid and ethanol. The ratios of the feedstock to solvent were 1:5, 1:15, and 1:25. The extraction was done at various microwave powers of 300, 450, and 600 W with extraction times of 3-15 minutes. The results indicated that the extraction of Cockspur Coral flowers with a ratio of feedstock-to-solvent of 1:15 gave the highest total anthocyanin content at a microwave power of 600 W for 3 min. Whereas, the highest total phenolic (132.73 g/L) was reached at the extraction with a microwave power of 600 W for 12 min. The method of extraction of Cockspur Coral flowers using a MAE method did affect the total phenolics content. However, this method apparently did not show a significant effect on the total anthocyanin content.

Keywords: Cockspur Coral flowers;anthocyanins;antioxidants; microwave-assisted extraction (MAE).

1. INTRODUCTION

Recently, natural dyes have become more popular in food, textile, and other industries compared to the synthetic dyes (Fröse et al., 2019; Souissi et al., 2018). This was because the synthetic dyes would rise specific problems to the environment and human health (Fajraoui et al., 2019). One of the safe natural dyes is anthocyanin.

Anthocyanin is a water-soluble compound that often exists in color-containing plants in Indonesia (Huang & Zhou, 2019). Anthocyanins could inhibit the growth of cancer cells, prevent cardiovascular diseases, control metabolic disorders, inhibit the age of nerve degeneration, and improve visual functions (Benvenuti et al., 2016). Anthocyanins could be found in the red parts of plants such as leaves, flowers, and fruits (Huang & Zhou, 2019) . One of the Indonesian plants which contains high

anthocyanin is Cockspur Coral flowers. Cockspur Coral (*Erythrina crista-galli*) has red flower petals that indicate the existence of anthocyanin pigment (Arita et al., 2014). Cyanidin-3-sophoriside in Cockspur Coral flowers was confirmed as a bioactive compound with antioxidant properties (Braga et al., 2019). Antioxidants in Cockspur Coral flowers were in the form of phenolic compounds, e.g. flavanone, chalcone, flavone, peterocarpan, isoflavanone, isoflavone, isoflavan, and isoflav-3-n derivatives. These compounds could act as an antimalarial, an anti-cancer, an anti-microbial and an antioxidant (Iqbal & Bhanger, 2006).

Anthocyanin could be extracted using a conventional solid-liquid extraction (CSLE), microwave-assisted extraction (MAE), and ultrasound-assisted extraction (UAE) methods (Da Porto & Natolino, 2018). In recent times, the microwave-assisted extraction (MAE) method has attracted a lot of interests because it gave many advantages, due to its high time efficiency, low level solvent use, and high yields of the target compounds (Liu et al., 2019; Llompart et al., 2019).

The extraction of a plant pigment using a microwave-assisted extraction method was mainly influenced some parameters, among others the type and ratio of feedstocks to solvents, microwave power, and extraction time (Camel, 2000; Sun et al., 2007; Ludin et al., 2018). Acidified alcohol solvents have been reported to be very effective for extracting anthocyanin from plants (Gao & Mazza, 1996). The addition of acids could improve the extraction results (Moulana et al., 2012). The use of ethanol in the extraction could allow the extracted anthocyanin to show the highest absorption spectrum (Liu et al., 2019; Arici et al., 2016; Díez et al., 2019). The addition of citric acid to alcohol solvents was reported to result in a higher total anthocyanin content and trigger a clearer pigment identification (Díez et al., 2019; Ludin et al., 2018). The optimum feedstock-to-solvent ratios were reported as 1:5 (Sun et al., 2007) 1:15 (Zou et al., 2012), 1:25 (Elİk et al., 2017). The MAE method was carried out at powers (W) of 300 (Abdel-Aal et al., 2014), 450 (Gala et al., 2018), 600 (Abdel-Aal et al., 2014) for 3-15 minutes (Sun et al., 2007; Pap et al., 2012; Liazid et al., 2011; Al Mamoori & Al Janabi, 2018). This study has investigated the effect of the ratios of feedstock to solvent, microwave power and extraction time in the microwave-assisted extraction of Cockspur Coral flowers.

2. METHODS

All of the chemicals used in this study were in analytical grade. Ethanol, citric acid, Folin-Ciocalteu reagents, Na_2CO_3, $CH_3COONa.3H_2O$ anhydrous, and KCl were purchased from Merck. Gallic acid was obtained from Sigma.

The Cockspur Coral flowers used in this study were obtained from Semarang city (Central Java, Indonesia). The flower petals were sorted, washed using distilled water and dried under sunlight. The dried Cockspur Coral petals were then ground and sieved with a size of 35 mesh. The powder of Cockspur Coral petals was stored in a closed container.

A 10 g of Cockspur Coral powder prepared in the previous step was extracted with 4% citric acid-ethanol with a ratio of feedstock to solvent of 1: 5. A Samsung MS23K3515AS microwave with a power of 300 W was used. The process was carried out for 3 min. After extraction, the filtrate was separated from the solid by filtration technique, followed by the distillation at 78^oC. The extract was dried in an oven at 78^oC to a constant weight. The experiments with various microwave powers (ranging from 450 to 600 W) and ratios of feedstock to solvent (1: 5, 1:15, and 1:25) at various extraction times (3, 6, 9, 12, 15 min) were performed with the same procedure mentioned above.

The total anthocyanin content in the extract of Cockspur Coral flowers was determined using a pH differential method as previously reported (Lee, 2005; Guisti & Wrolstad, 2001). A Eutech Instruments pH meter was used in the pH measurement. A 0.1 g of the extract was dissolved in distilled water until the volume was 10 mL. Two cuvettes were prepared and each was added with 0.2 mL of the sample. The first cuvette was added with 0,8 mL of KCl buffer (1.864 KCl in 960 mL distilled water) to adjust the pH of the sample to a pH of 1.0. The second cuvette was added with 0.8 mL of $CH_3COONa.H_2O$ buffer (32.814 g CH_3COONa in 960 mL distilled water) to adjust the pH of the sample to pH of 4.5. The addition of a few amount of hydrochloric acid solution was allowed to have a precise value of pH. The absorbance of each

sample was measured using an analytical scale Genesys 10 UV-Vis spectrophotometer at wavelengths of 515 and 700 nm. The final absorbance of both samples with different pH was calculated using equation (1), where A_{515} and A_{700} referred to the absorbance of the sample with the corresponding pH at 515 and 700 nm, respectively. The total anthocyanin content in the extract sample could be calculated by using equation (2), where A is the final absorbance calculated using equation (1), MW is the molecular weight of cyanidin-3-glucoside (449.2 g/mol), Vd is final dilution volume (L), ε is the cyanidine-3-glucoside absorbancy molar coefficient (26,900 L/mol/cm), and l is the length of the cuvette (cm).

$$A = (A_{515} - A_{700})pH_{1.0} - (A_{515} - A_{700})pH_{4.5} \quad ...(1)$$

$$Anthocyanin\ \left(\frac{mg}{L}\right) = \frac{A}{\epsilon x\, l}\ x\ MW\ x\ \frac{Vd}{1000} \quad ...(2)$$

The total phenolics content of the Cockspur Coral flower extract was determined by using Folin-Ciocalteau reagent as previously reported (Kumar et al., 2014). A 1 mL of Cockspur Coral flower extract was added in the mixture of 2.5 mL of 50% (v/v) Folin-Ciocalteau reagent and 7.5 mL of Na_2CO_3 7.5% (w/v). The mixture was incubated for 30 minutes. This mixture was designated as "sample". The absorbance of gallic acid solutions with various concentrations was measured at a wavelength of 765 nm. The absorbance of the sample was measured with the same procedure as gallic acid solutions. The sample concentration was calculated by using the linear regression equation obtained for the gallic acid standards, y = ax + b, where y is the absorbance of the sample and x is the gallic acid concentration of the calibration curve. The total phenolic content contained in the extract was then calculated using equation (3), where C is the total phenolic content (mg GAE/g extract), C_1 is the concentration of gallic acid from the calibration curve (mg/mL), V is the extract volume (mL), and m is the weight of extract (g).

$$C = \frac{C_1\ x\ V}{m} \quad ...(3)$$

3. RESULTS AND DISCUSSION

3.1. Total Anthocyanin Content in the Cockspur Coral Flower Extract

The content of total anthocyanin in the Cockspur Coral flower extract with 4% citric-ethanol solvent with feedstock-to-solvent ratios of 1:5, 1:15, and 1:25 is presented in **Figure 1**.

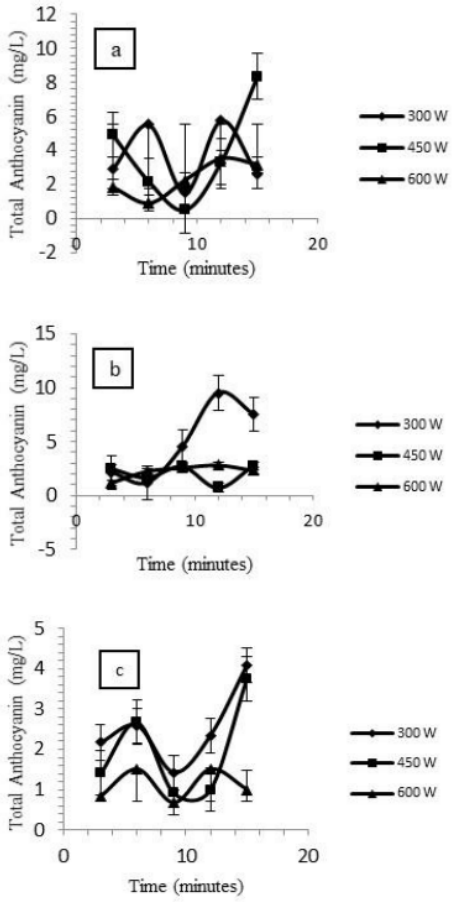

Figure 1. Total anthocyanin content of the Cockspur Coral flower extract in 4% citric acid-ethanol solvent using a ratio of feedstock to solvent of a) 1:5, b) 1:15, and c) 1:25.

Figure 1 indicated that a microwave power of 450 W for 15-min extraction time resulted in a high concentration of anthocyanin i.e. 8.349 mg/L, as is shown in Figure 1a. Moreover, the highest anthocyanin concentration (9.518 mg/L) was achieved by the extraction with a microwave

power of 300 W for 12 min as is shown in Figure 1b. A 300 W microwave power for 15-min extraction time could produce 4.091 mg/L of anthocyanin. This was in line with that reported earlier (Song et al., 2011). The use of microwave power was believed directly proportional to the extraction temperature (Alara et al., 2019). An increase in the extraction temperature could increase the solubility of anthocyanin in the solvent and further decrease its viscosity (Liu et al., 2019). The characteristics of the feedstock could show a great effect on the extraction process. It was possibly the reason why the highest power used in the extraction (600 W) did not necessarily produce the best anthocyanin concentration (Kusuma et al., 2017). It was suspected that the use of high microwave power (e.g. 450 W and 600 W) could lead to degradation of the starting material (Li et al., 2012).

In general, the anthocyanin concentration that could be recovered from the extraction with a feedstock-to-solvent ratio of 1:5 (Figure 1a) was not significant with a fluctuation trend. This phenomenon was also observed in Figure 1b and 1c. A longer extraction time could result in an insignificant increase in the extract yield (Kusuma et al., 2017). Apparently, all the anthocyanin in the feedstock has already been extracted during the employed extraction time. Also, It was possibly that the feedstock contained a certain amount of anthocyanin. The anthocyanin was possibly already dissolved by the solent and entirely extracted from Cockspur Coral flower (Anbalagan et al., 2019).

The optimum anthocyanin content produced from Cockspur Coral extract using conventional methods produced 0.0843 mg anthocyanins/10 g feedstock at 3 hours (Purwanti & Parjoko, 2016). Meanwhile, the highest yield of anthocyanin obtained by the use of MAE method with a ratio of feedstock to solvent of 1:25 and a 300 W microwave power for 15 min was 4.091 mg/L. Heating with conventional methods would require a longer time for the powder material to diffuse into the solvent due to the absence of internal strength pore (Daniswara et al., 2018). Using microwaves in the extraction process would improve the diffusion rate of the solvent to the feedstock components (Lin et al., 2017).

The P-value of the total anthocyanin content was analyzed by using a single factor analysis technique to determine the validity of the data obtained. The P-values were 0.491, 0.091, and 0,092 for Figure 1a, b, and c, respectively. If the P-value was less than α (α = 0.05), the data obtained was significant. In contrast, if the P-value was more than α, the data obtained was not significant. These p-values indicated that the anthocyanin data obtained was not significant.

3.2. Total Phenolic Content in the Cockspur Coral Flower Extract

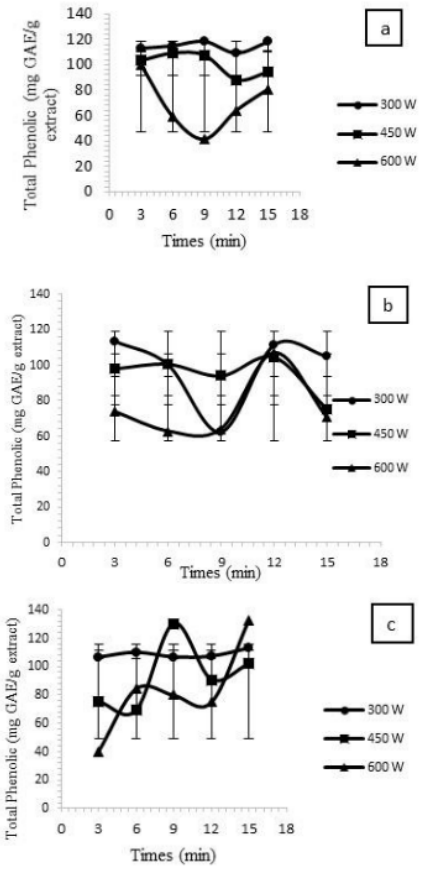

Figure 2. Total phenolics in the Cockspur Coral flower extract in 4% citric acid-ethanol solvent with a ratio of feedstock to solvent of a) 1:5, b) 1:15, and c) 1:25.

The total phenolic content was determined using Folin-Ciocalteu (FC) reagent. The Folin-Ciocalteu (FC) reagent contained phosphomolybdate-phosphotungstate which will oxidize the hydroxyl groups from phenol compounds to produce blue

complexes (Opitz et al., 2014). The changes in the antioxidant activity with time (3-15 min) at various ratios of feedstock to solvent in the extraction of Cockspur Coral flower using MAE method is presented in **Figure 2**.

The highest total phenolics (118.933 g/L) was achieved in the extraction of Cockspur Coral flowers using a 300 W microwave power for 12 min, as is shown in **Figure 2a**. The highest total phenolic of 113.418 g/L was achieved in the extraction of Cockspur Coral flowers using a 300 W microwave power for 3 min (**Figure 2b**). The highest ratio of feedstock to solvent of 1:25 using a 600 W microwave power for 15 min gave a total phenolic of 132.729 g/L.

A fluctuative trend of total phenolics was observed in **Figure 2**. The same phenomenon was reported previously (Alara et al., 2019). Apparently, an improper choice of microwave power levels and solvent concentrations could lead to less precise the phenolic content (Kusuma et al., 2017). Microwaves could affect the content of antioxidants obtained in the MAE process. A higher microwave power could result in a decrease in the total antioxidant content (Alara et al., 2019). Moreover, the increase in the microwave power could accelerate the movement of solvent molecules, the breakdown of feedstock, and extractive diffusion into solvents (Abdel-Aal et al., 2014). However, an excessive microwave power (> 400 W) could cause a degradation of some antioxidant compounds in the feedstock (Hu et al., 2017).

The total phenolics for **Figures 2a, 2b**, and **2c** were analyzed by P-value using a single factor analysis technique to determine the validity of the data. If the P-value <α (α = 0.05), the data obtained was significant, while the P-value> α, the data obtained was not significant. The P-values for Figures 2a, 2b, and 2c were 0.00074, 0.123 and 0.254, respectively. This showed that **Figure 2a** gave a P-value <0.05, indicated that the data obtained was significant. In contrast, **Figure 2b** and **2c** gave a P-value> 0.05 indicating that the data obtained were not significant.

4. CONCLUSIONS

The extraction of Cockspur Coral flower using a microwave-assisted extraction method could gave 9.518 mg/L of total anthocyanin content at a power of 300 W for 12 min. Moreover, the microwave-assisted extraction of Cockspur Coral flower with a power of 600 W for 15 min could gave total phenolics of 132.729 g/L.

5. ACKNOWLEDGEMENT

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