Anthocyanin Content of Petal of Red Rose (*Rosa Damascene* Mill.) and Red China Rose (*Hibiscus Rosa-Sinensis* L.) from Maceration and Percolation Method

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ABSTRACT

Aim: The aim of this study was to compare the total anthocyanin content of petals of red rose and red China rose which extracted by maceration and percolation method.

Method: Total anthocyanin content was determined by pH differential method, due to structure alteration of anthocyanin depends on pH.

Results: Total anthocyanin content of macerate of red rose and red China rose was 0.459 ± 0.003 mg/L and 0.186 ± 0.006 mg/L, respectively. Total anthocyanins content of percolate of red rose and red China rose was 0.366 ± 0.005 mg/L and 0.078 ± 0.002 mg/L, respectively.

Conclusion: Total anthocyanin content was affected by botanical family and extraction method.

Keywords: extraction method, pH differential, pH dependence, color alteration

INTRODUCTION

Red rose (*Rosa damascene* Mill.) of Rosaceae family has sencondary metabolites, such as terpenes, glycosides, flavonoids, and anthocyanins which isolated from flowers, petals and hips [1-4]. The pharmacological function of Rosaceae, i.e. antioxidants, free-radical scavengers [5], anticancer [6], antimutagenic [7], antidepressant [8], and anti-inflammatory [9], are attributed to phenolics compounds.

Red China rose (*Hibiscus rosa-sinensis* L.) of Malvaceae family contain vitamins, flavonoids, ascorbic acid, niacin, riboflavin, thiamine and cyanidin diglucoside (anthocyanins) in flowers [10]. Its flowers have pharmacological function, such as oral contraceptive, laxative, aphrodisiac, [11].

The petals of red rose and red China rose are red because of anthocyanins, water-soluble plant pigments, which can be used as natural dye and herbal medicines due to secondary metabolites. Anthocyanins color stability depend on anthocyanins structure, pH, temperature, oxygen, light, and water activity [12]. The aim of this study was to compare the total anthocyanin content of petals of red rose and red China rose which extracted with maceration and percolation method.

MATERIALS AND METHODS

Materials

Red roses and red China roses petals were collected from Manoko Garden, Lembang Subdistrict, West Java, Indonesia. The plants were identified at Laboratory of Plant Taxonomy, Department of Biology, Universitas Padjadjaran with No.561/HB/02/2018. All chemicals are analytical grade (Merck, Germany).

Loss on Drying

Red rose and red China rose petals were weighed and dried on 105°C at atmospheric pressure for 5 h, then weighed. Drying and weighing continued with 1 h interval, until a constant weight [13].

Anthocyanins Extraction and Identification

Each petals were extracted with acidic solvents, i.e. 96% ethanol and 2 N hydrochloride acid pH 1.0 with a ratio 1:20. Maceration and percolation were conducted for 24 h at ambient temperature. All extract were filtered and centrifuged, then transferred to 100 mL of volumetric flask [14]. Each extract was added with 2 M hydrochloride acid, then heated at 100°C for 5 min. The extract color was still red (a). Each extract was added with 2 M sodium hydroxide. The color alteration was observed from red to blue-green, then vanished (b) [15].

Analysis of Total Anthocyanin Content

Modified pH differential method [14] was used to determine the total anthocyanins content. The dilution factor was 15.3 for red rose and 12.0 for red China rose.

Statistical Analysis

The results were presented as mean ± standard deviation (SD). Oneway ANOVA followed by t-Student test, with considered statistically significant at $p < 0.05$, were used to statistical analysis.

RESULTS AND DISCUSSION

Results of Loss on Drying

Loss of drying of red rose and red China rose petal was 4.2% and 4.1%, respectively, which met the requirement [16]. It should be determined due to affect the calculation of total anthocyanin content. Loss of drying that do not meet the requirements cause low concentration of secondary metabolites due to degradation by hydrolysis or enzymatic reactions.

Results of Anthocyanins Extraction and Identification

Maseration and percolation were chosen due to cold extraction methods can mantain the anthocyanins stability which decompose on heating. It was indicated by the color alteration of anthocyanins from red to colorless [19]. The time of anthocyanin extraction with cold method has been optimized (data not shown). The optimal time for anthocyanin extraction was 24 h. The length of extraction time was not proportional to the anthocyanins content. It was caused by anthocyanins decomposed easily in solution [19].

A polar solvents are used to anthocyanins extraction [15], such as water (1.00), methanol (0.76), and ethanol (0.56) [17]. Highest total anhocyanin content was methanolic extract pH 1.0, followed by ethanolic extract pH 1.0 and aqueous extract pH 1.0 [18]. Methanol is more toxic

than ethanol, so ethanol used as a solvent for anthocyanin extraction. Anthocyanins are most stable at pH 1.0 [19], so acidic ethanol pH 1.0 was chosen as a solvent. All extract color were red, due to red oxanium form of anthocyanin [19].
All extracts were filtered to separate extract from simplicia. Very fine simplicia can passed through the filter paper, so extracts need to be centrifuged. Anthocyanins have good solubility in acidified ethanol [15, 18] so it won't sedimented. Centrifugation produces a clear supernatant so it can be applied to measurements with a visible spectrophotometer. The turbid extract will reduce anthocyanins absorption because visible light is blocked by dissolved particles [19]. All extract contain anthocyanin, due to color alteration in acidic and alkaline solutions, i.e. anthocyanins is green to yellow in an alkaline solution and purple to red in acidic solution [20].

Results of Analysis of Total Anthocyanin Content

Tabel 1 Total Anthocyanin Content

Simplicia	Total anthocyanins content (mg/L) from	
	Maceration	Percolation
Red rose petals	0.459 ± 0.003	0.366 ± 0.005
Red China rose petals	0.186 ± 0.006	0.078 ± 0.002

Total anthocyanin content was determined by pH differential method, due to pH influence the anthocyanin form, at pH 1.0 is red oxanium form and at pH 4.5 is colorless hemicetal form [19]. Total anthocyanin content of red rose was higher than red China rose (Table 1), due to different botanical family. Rosaceae family has higher total anthocyanin content than the Malvaceae family. This can be observed visually from the petals color. Red rose petals were redder than red China rose petals. Total anthocyanin content was significantly different for differences in botanical family, i.e. 9.40×10^{-8} for maceration method and 5.76×10^{-8} for percolation method.
In maceration, the simplicia was soaked for 24 h in the solvent with stirring every 2 h. In percolation, the simplicia was soaked for 4 h in the solvent, then the solvent was passed through simplicia for 20 h. At a ratio 1:20, it can be observed that total anthocyanin content of macerate was higher than percolate (Table 1). It was caused by contant time between solvent and simplicia of maceration method was longer than percolation. Total anthocyanin content was significantly different for extraction method, i.e. 6.52×10^{-6} for red rose petals and 3.06×10^{-9} for red China rose petals. It was concluded that extraction method affect total anthocyanin content.

CONCLUSION

Total anthocyanin content was affected by botanical family and extraction method.

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