Chemical Analysis of Iron, Vitamin C, Tannins and Total Flavonoids in Ethanol Extract of Jamaican Cherries Fruits (*Muntingia calabura L*) for Development of Antianemia

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Abstract: Various natural sources such as red guava, moringa leaves and snake fruit seeds have been developed for anemia treatment. Fresh Jamaican cherries is known to have 178.96 mg / 100 g vitamin C of and 0.10 mg / 100g Fe. Flavonoids, especially chrysin and quercetin are bioactive compounds, which have an important role in improvement of haemoglobin (Hb) levels. The aim of this study was to extract Jamaican cherries fruits and to analyse that extract in terms of vitamin C and iron concentrations, total flavonoids and tannins. Ripe fruits of Jamaican cherries were collected from Sleman, Yogyakarta and dried cherries were extracted using the maceration method with ethanol solvent. Chemical analysis of vitamin C and flavonoids was carried out at the Integrated Laboratory of Universitas Ahmad Dahlan while Fe and tannins concentrations were analysed at the Food and Nutrition Research Centre, Universitas Gadjah Mada, Yogyakarta. We got 34% ethanol extract of Jamaican cherries fruits. The highest chemical concentration was tannins (2.96 ± 0.01%), followed by Fe (0.104 ± 1.05%), vitamin C (0.09 ± 0.01%), and total flavonoids (0.0012 ± 0.56%). In conclusion, ethanol extract of Jamaican cherries fruits becomes a promising nutraceutical for anemia treatment in future.

1 INTRODUCTION

Iron deficiency anemia persists a nutritional health problem in the world by which becomes one of the main causes of Global Disability Adjusted Life Years (DALY's) in adolescents (Vos *et al,* 2020). Iron fortification and supplementation are recommended to overcome this problem. However, some adolescents with anemia have minor responses to those treatments.

Medicinal plants are a very popular alternative treatment in some countries. Data from WHO (2018) have indicated that 85% African and Southeast Asian countries, 63% western Pacific regions and 43% eastern Mediterranean regions use traditional medicine for treatment of human diseases (WHO, 2019). Iron deficiency anemia is one of human diseases, which can be treated using natural sources such as red guava, moringa leaves or snake fruit seeds extract. However, research evidence is still required to figure out efficacy and safety for long term use (Yusnaini, 2016; Arini, 2018; Ristanti, 2019).

Jamaican cherries (*Muntingia calabura L*) for example, it easily grows in any type of soil in Indonesia and produces its fruits in all seasons (Ningsih, 2016). Many people believe that the Jamaican cherries have beneficial effects for human health (Mahmood *et al*., 2014). Previous studies have reported jamaican cherries extracts derived from roots, leaves and barks as alternative treatments for diabetes, microbial infection and inflammation. Generally, 100g fresh fruits of Jamaican cherries contain 178.96 mg vitamin C and 0.10 mg Fe

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(Makahity *et al*., 2019). In addition, 1g dried fruits of Jamaican cherries consists of flavonoids, which are equal to 1.84-21.80 Myricetin Equivalents (ME) (Nurholis & Saleh, 2019). Unripe and ripe fresh fruits of Jamaican cherries also contain large amounts of flavonoids such as rutin, myricetin, luteolin, quercetin, apigenin, and kaempferol (Kubola *et al*., 2011). Some studies have used flavonoids for anemia treatment (Borawska *et al*., 2014) and a recent study reported that administration of 50 mg / Kg BW quercetin increases hemoglobin and serum iron levels in mouse model with anemia (Mazhar *et al*., 2018).

Some previous studies related to extraction of Jamaican cherries fruits have been done. For example, Gomathi *et al* (2012) reported the extraction of Jamaican cherries fruits using a mixture of methanol, acetone and water. Meanwhile, Novita (2016) extracted Jamaican cherries fruits with ethanol or waters solvent. In addition, Subagya (2019) extracted Jamaican cherries fruits with 70% ethanol for anti-bacteria and Sujono *et al* (2021) reported the extraction of Jamaican cherries fruits using a ethyl acetate or methanol solvent for identification phytochemical profile. From some research studies, they did not measure Fe, total flavonoids and tannin levels. In addition, only Novita's study has measure vitamin C level in the extract but it used the iodometric titration method. Therefore, the aim of this study was to extract Jamaican cherries fruits with ethanol solvent and to analyse that extract in terms of vitamin C, iron, total flavonoids and tannins concentrations.

2 MATERIALS AND METHODS

2.1 Jamaican Cherries Extraction

Ripe Fruits of Jamaican cherries were collected from street side in Ambarketawang, Sleman, Yogyakarta for around 2 months. Washed Jamaican cherries were then dried using cabinet dryer at 60^0C for 26 hours and then were grounded using a grinder with a 80 mesh sieve at Rumah Berkah Herbal Group in Yogyakarta.

Jamaican cherries fruits extracted with the existing maceration method by Ristanti (2019) for extraction of snake fruit seeds. A total of 300g flour of Jamaican cherries was extracted using the maceration method with 1.5L 70 (volume/volume) % ethanol (p.a merck, Germany) at room temperature (28^0C ± 2^0C) for 3 days. Furthermore, the solution was filtered using 40µm Whatman filter paper to separate the residue and filtrate. Furthermore, the residue was re-macerated using the same solvent with 1:3 ratios for 2 days at room temperature. The re-suspended solution was filtered again using the 40 µm Whatman filter paper to obtain the second filtrate. The first filtrate was combined with the second filtrate and then was concentrated with a rotary vacuum evaporator (Rotavapor R215) at 60^0C, 60 rpm and 175 mbar. Finally, Jamaican Cherries extract was kept at refrigerator until further analysis.

2.2 Determination of Extract Yield

The yield of Jamaican cherries fruits extract was calculated using the following formula:

$$\% \text{ Yield} = \frac{Pasta\ amount\ (g)}{Simplicia\ weight\ (g)} \text{ x } 100\%$$

2.3 Micronutrient Analysis

Measurement of vitamin C and total flavonoid levels in Jamaican cherries fruit extract was carried out at the Integrated Research Laboratory, Faculty of Pharmacy, Universitas Ahmad Dahlan, Yogyakarta while Fe and tannins levels were analysed at the Laboratory of the Center for Food and Nutrition Studies, Universitas Gadjah Mada. 100 mg Jamaican cherries fruit extract was dissolved in distilled water and were then analysed using the UV-Vis spectrophotometer with 200 - 400 nm wavelength (Makahity *et al*., 2019).

For measurement of Fe levels, 25 mg Jamaican cherries fruits extract was dissolved in distilled water. A total amount of 0.1 ml dissolved extract was reacted with 1.1 ml of 100 ppm $Na_2S_2O_3$, 1.10 ml of 1,000 ppm phenanthroline solution and 1.5 ml acetate buffer pH 4.5. After that, 5 ml of acetone and distilled water was added into the above solution and was incubated at room temperature for 120 minutes. The solution was analysed using the UV-Vis spectrophotometer with 400 - 800 nm wavelength (Makahity *et al.*, 2019).

2.4 Determination of Total Flavonoids and Tannins

To determine total flavonoids, 25 mg Jamaican cherries fruits extract was dissolved in 25 ml of 96% ethanol. Then, 1 ml dissolved extract was reacted with 3 ml of 96% ethanol, 0.2 ml $AlCl_3$ and 0.2 ml sodium acetate. Furthermore, it was spectrophotometrically measured at 430 nm wavelength (Winahya *et al*., 2018). Meanwhile, tannins measurement used 1 mg Jamaican cherries

fruits extract, which was dissolved in distilled water to reach 10 ml (100 ppm). After that, 9 ml the solution was re-dissolved in distilled water until 10 ml (90 ppm). Next, the solution was mixed with 1 ml folin-denis' reagent for 3 minutes. The mixed solution was then added with saturated Na_2CO_3 and was incubated for 40 minutes. Furthermore, it was spectrophotometrically measured at 650 nm wavelength (Pratama *et al*., 2019).

3 RESULTS AND DISCUSSION

In the Table 1, we have extracted local Jamaican cherries fruits with the existing maceration method for extraction of snake fruit seeds (Ristanti, 2019). Previously, we also calculated the yield value of Jamaican cherries fruit flour to see the weight loss of Jamaican cherries fruit due to the drying process. The high water content of Jamaican cherries fruit a low flour yield. The yield of this method extraction was 34 (weight/weight)%, which was slightly greater than another previous study (32.68%).

Table 1: The Yield of Jamaican Cherries Extract.

Sample	Yield (%)
Jamaican Cherries Fruits Flour	0.14
Jamaican Cherries Fruits Extract	34

The difference in the extract yield was caused by maceration time and frequency. In our study, we macerated Jamaican cherries fruits for 3 days while Subagya study macerated them for 2 days. In addition, the residue of Jamaican cherries was re-macerated for 2 days. A research conducted by Subagya, Jamaican cherries fruits extract was used to inhibit *Porphyromonas gingivalis* proliferation (Subagya, 2019) while our study used the ethanol extract of jamaican Cherries for iron suplementation.

In this present study, we have firstly documented the iron level in the ethanol extract of Jamaican cherries fruits. Table 2. indicated micronutrients and phtochemicals analysis in the ethanol extract of Jamaican cherries fruits. The highest level of chemical compounds in the extract was tannins (2.96 ± 0.01%), followed by Fe (0.104 ± 1.05%), vitamin C (0.09 ± 0.01%), and total flavonoids (0.0012 ± 0.56%). Because we used a polar solvent, it is not surprising that our extract contains tannin. The result of tannin analysis in our study is accordance to other studies (Krishnaveni & Dhanalakshmi, 2014 ; Hadi & Permatasari, 2019), but their studies only used a qualitative method. Tannins are an important chemical compound in the most plant kingdoms because of their role for predator protection and regulatory growth (Das *et al*., 2020).

There is a limited study that reported a disadvantage effect of tannin for inhibition of Fe absorption. Based on Greger *et al* (1988), administration of tannic acid or black tea consumption impairs iron bioavailability and status, in anemia rats. In contrast, administration of condensed tannins (proanthocyanidins) and Soybeen seed ferritin (SSF) has slightly different effects of ferritin and iron serum levels in rats with anemia, compare to rats with anemia treated with SSF only (Yun *et al*, 2011). In addition, epidemiological studies and long-term trials show that individual iron status is not affected by tannin consumption because the human body has the adaptation mechanism such as chloric acid in gaster and the present of vitamin C from daily intake to minimize the anti-nutritional effect of tannins (Delimont *et al*, 2017). Due to the high present of vitamin C in our extract, we believe that the anti-nutritional effect of tannin will be neutralized by vitamin C although we need further investigation.

Table 2: Results of Vitamin C, Fe, Total Flavonoids and Tannins Levels of Ethanol Extract of Jamaican Cherries Fruits.

Chemical Analysis	Mean ± SD (%)
Tannin	2.96 ± 0.01
Fe	0.104 ± 1.05
Vitamin C	0.09 ± 0.01
Total flavonoids	0.0012 ± 0.56

Our research finding showed that iron is the second highest level of chemical compounds in the ethanol extract of Jamaican cherries fruits. This result of the present study is different from a previous study by Makahity *et al* (2019). They reported that 100g fresh Jamaican cherries fruits were collected from Air Louw, Nusaniwe, Ambon contains 0.10 mg iron, 343,39 mg carbohydrates, 178,96 mg vitamin C and 1,48 β-carotene. Using the same extraction method, Ristanti (2019) reported that ethanolic extract of snake fruit seeds has higher iron level (211 mg/100g) than simplicia of snake fruits seeds (26.88 mg/100 g). Therefore, this shows that the extraction process using 70% ethanol solvent can dissolve iron effectively. It potentially becomes an important source of iron for anemia treatment.

In contrast to tannin and iron levels, vitamin C in the ethanol extract of Jamaican cherries fruits is lower than the fresh Jamaican cherries fruit (178.96 mg /

100 g), reported by Makahity (2019). The decreased level of Vitamin C in our extract is due to the drying process that using a constant temperature (60^0C) for 26 hours. The longer heating time is associated with the higher degradation of vitamin C (Igwemmar *et al*., 2013). Furthermore, our research finding of vitamin C level in the Jamaican cherries fruits extract is higher than that of Novita study (2016) which found that the vitamin C level in her extract was 17.64 mg/100 g. The discrepancy of different vitamin C levels may be caused by the different analytical method. We used Uv-Vis spectrophotometrical analysis while she used the iodometric titration method, which is less accurate.

The total flavonoids levels in Jamaican cherries fruits have been reported by some researchers. According to Senet *et al* (2017), they found that total flavonoids level in fruits of Jamaican cherries ethanol extract are 134.4 mg/100 g and 29.9 mg/100 g of Jamaican cherries ethyl acetate extract, higher than total flavonoids level in our study (12.25 ± 0.56 mg/L). The huge differences in flavonoid levels result from the pre-extraction process. The fresh fruits of Jamaican cherries were firstly soaked for 9 days and directly macerated gradient maceration method using n-hexane, ethyl acetate, and ethanol as solvents with 2 litres of solvent replacement every 3 days. The longer time for soaking will get the higher amount of phytochemicals during maceration process (Wijaya *et al*., 2018).

Recently, the total flavonoids level is considered as a new perspective for anemia treatment. Quercetin and chrysin for instance, has been administered to mouse model with anemia and normal rats. Administration of 50 mg / Kg BW quercetin and 50 mg / Kg BW $FeSO_4$ in rat with anemia significantly increase haemoglobin and serum iron levels for 30 days, compared to normal and anemic groups treated with $FeSO_4$ only (Mazhar *et al*., 2018). In addition to quercetin, administration of 100 mg/Kg BW chrysin was reported to increase Hb levels in normal rats for 7 days, compared with the control group (Borawska *et al*., 2014). However, the limitation of our study is we did not quantify the quercetin and chrysin levels in our extract.

4 CONCLUSION

Extraction of Jamaican cherries fruits using 70% ethanol yields 34% extract. The ethanol extract of Jamaican cherries fruits has 0.104% Fe level which becomes a promising nutraceutical for anemia treatment. However, further studies are required to analyse quercetin and chrysin levels in ethanol extract of Jamaican cherries fruits and to figure out the synergetic effect of those flavonoids and Fe level to increase haemoglobin level in anemia rats model.

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