

A Study of Antioxidant Activities in Guava Fruit: Solvents Effect

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Abstract—Guava has some potential antioxidant compounds that are varied in characteristics and solubilities. In this research, the study of solvent (methanol, ethyl acetate, and hexane) effects on extraction process of antioxidant compounds in guava fruit were conducted. The extraction method used was sonicator for 1 hour. Determination of antioxidant activity of guava fruit was carried out using DPPH assay. The result showed that extraction yields on different solvents were varied. Methanol extract has the highest extraction yield while the smallest yield was hexane. However, in DPPH assay, the highest antioxidant activity was showed in ethyl acetate extract. The solvent polarity in extraction process was very influential on antioxidant activity.

Keywords: guava, solvent, antioxidant, DPPH

I. INTRODUCTION

Guava is one of the natural antioxidant resources that is widely available. Antioxidants are substances which retard or prevent the formation of free radicals. If free radicals react with the biological molecules, it can cause some degenerative diseases such as arteriosclerosis, cancer, and Parkinson's [1], [2].

Some research stated that guava has an antioxidant activity. Antioxidant activity of guava is higher than mango, dragon fruit, banana (mas), water apple, orange, and star fruit [3], [4]. Reference [5] reported that guava juice had a high antioxidant capacity. This activity caused by the potentially antioxidant compounds of guava fruit.

Ascorbic acid and some phenolic compounds such as apigenin, myricetin, and anthocyanins are several bioactive compounds in guava [6], [7]. Furthermore, it contains some carotenoids e.g. β -carotene and lycopene that are potentially antioxidant compounds [8].

Solvent polarities, used for extraction process, are very influential on the type of bioactive compounds that can be extracted. Polar solvents (i.e. water and methanol) are used to extract hydrophilic antioxidant compounds (i.e. ascorbic acid and phenolics) On the other hand, non-polar solvents (hexane and petroleum ether) are used to extract lipophilic antioxidants compounds (i.e. carotenoids) [9], [10].

Organic solvents such as ethanol, methanol, ethyl acetate, diethyl ether, and hexane have been usually used in extraction process. Some antioxidant compounds from plants and fruits, such as *Moringa oleifera* leaves, papaya, and mango can be extracted by methanol [11], [12]. Antioxidant compounds in Kintamani orange and Jember orange can be

extracted by ethyl acetate [13]. Hexane can extract antioxidant compounds of nutmeg [14].

This research was conducted to investigate the effect of solvent polarities on the extraction process to the antioxidant activity of guava fruit. Based on literature study that have been carried out, there were no data showed the antioxidant activity of guava fruit which extracted by non-polar and semi polar solvent. The solvent used were methanol, ethyl acetate as a semi polar solvent [15], and hexane as a nonpolar solvent [16]. Extraction process was conducted by sonification and DPPH assay was used to antioxidant activity determination.

II. METHODOLOGY

A. Material

Pink flesh guava fruit (*Psidium guajava*, Linn) obtained from the traditional market in Purwokerto was used in this research. Vitamin C (PI 50 mg) was from PT. Supra Ferbindo Farma. Methanol p.a., Ethyl acetate p.a., and hexane p.a. were purchased from Smartlab. DPPH (2,2-diphenyl-1-picrylhydrazyl) was bought from Sigma-Aldrich.

Analytical balance (KK-LAB, Kenko), UV-Vis Spectrophotometer (Biobase), sonicator, waterbath (Mettler), rotary evaporator (Biobase), vortex, centrifuge, and blender (Maspion) were used.

B. Sample Preparation and Extraction

Fresh guava fruit were washed and blended to a uniform slurry without peeling the skin. All slurry samples (1 g) and 10 mL solvent (methanol p.a., ethyl acetate p.a., and hexane p.a.) were extracted using sonicator at room temperature. The extraction process was conducted for 1 hour. Cloudy samples were centrifuge for 10 minutes at 3,000 rpm. Clear supernatans were collected and evaporated at 50-65 °C.

C. Antioxidant Activity

2 mL of methanolic DPPH solution (0,1 mM) was added to 2 mL of methanolic guava extract solution (1-500 ppm). At room temperature, the mixture was incubated in dark conditions. UV-Vis Spectrophotometer was used to determine the antioxidant activity by measuring the absorbance of the mixture at 515.5 nm. The absorbance value then entered to this formula:

$$\text{Inhibitory activity (\%)} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100\% \quad (1)$$

III. FINDINGS AND DISCUSSION

A. Solvent Extraction Yields

Amounts of the guava slurry was extracted using three different solvents, methanol as a polar solvent, ethyl acetate as a semi polar solvent, and hexane as a non-polar solvents. Table 1 showed the extraction yields of different solvent extraction.

The result showed that extraction solvents affect the extract yields. Methanol had the highest extractable components, followed by ethyl acetate and hexane. Chemical compounds in plants have varied characteristics and solubilities in a different solvent. Therefore, the extractable components are different from one solvent to others [17]–[19].

TABLE I. EXTRACTION YIELDS OF DIFFERENT SOLVENTS

Name	Yields	Characteristics	
		Form	Colour
Methanol extract	3.928%	Thicky	Brownish green
Ethyl acetate extract	0.171%	Thicky	Brownish yellow
Hexane extract	0.049%	Thicky	Orange

B. Antioxidant Activity in Different Solvent

Antioxidant activity of guava fruit was investigated using DPPH method based on the hydrogen donor of antioxidant compounds to the DPPH radical (DPPH•). The antioxidant activity is proportional to the DPPH radical (DPPH•) disappearance. It can be monitored by naked eye (from purple to yellow color) or by UV-Vis spectrophotometer for accuracy.

Antioxidant activity of guava extract can be showed with IC₅₀. The lower IC₅₀, the higher antioxidant activity. Antioxidant activities of three guava extract are listed below.

TABLE II. ANTIOXIDANT ACTIVITY OF GUAVA EXTRACT IN THREE DIFFERENT SOLVENT

Extract	IC ₅₀ (ppm)
Vitamin C (standard)	4.360
Methanol	195.147
Ethyl acetate	81.915
Hexane	-

Ethyl acetate extract showed higher antioxidant activity (81.915 ppm) than methanol extract (195.147 ppm). Based on the IC₅₀, it can be classified that ethyl acetate extract of guava fruit has a strong antioxidant activity, while methanolic extract of guava fruit has a weak antioxidant activity [20]. This research has similar result with *mengkudu*. Ethyl acetate extract of *mengkudu* fruit has higher antioxidant activity than methanolic extract [21].

In this study, hexane extract of guava fruit has no IC₅₀. It can be caused by no potential antioxidant

compound extracted with hexane. Carotenoids in guava fruit can be extracted with *tert*-butyl methyl ether (*t*-BME) followed by ethyl acetate and methanol in high-performance mixer [8].

IV. CONCLUSION

Solvent, used in extraction, were very influence to the antioxidant activity of guava fruit. Polarity of the solvent has a high effect on the extractable potential antioxidant compounds. The ethyl acetate extract of guava fruit has a strong antioxidant activity with the lowest IC₅₀. For further study, it needs to prove the effectiveness of antioxidant activity in animal.

ACKNOWLEDGMENT

The authors would like to thank to The Dwi Puspita Foundation and The Harapan Bangsa University for the for the financial assistance and laboratory facilities.

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