

Identifying Antioxidant Activities of Guava Fruit Using DPPH Method

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Abstract—Antioxidant plays an important role in preventing degenerative diseases. One of fruits that has a potential antioxidant activity is guava. This research was aim to identify antioxidant activity of guava fruit. Potential antioxidant compounds in guava were extracted using sonicator, as a green extraction technique. DPPH (2,2-diphenyl-1-picrylhydrazyl) was used to determine antioxidant activity. Vitamin C was used as a reference standard. The result showed that ethyl acetate extract of guava fruit has a high antioxidant activity. The presence of flavonoids, steroids, and tannins in ethyl acetate extract were predicted as a potential antioxidant compounds.

Keywords: antioxidant, DPPH, green extraction, guava

I. INTRODUCTION

Free radical is a reactive molecule that contain one or more unpaired electrons and initiating a chain reaction. Free radicals are beneficial to human body in moderate level. In high concentration, free radicals can damage the vital molecule in the body, such as DNA, carbohydrates, lipids, and proteins. These can cause some degenerative diseases i.e. cancer, diabetes mellitus, hypertension, heart failure, cataract and retinal disease, renal failure, Alzheimer's, etc [1], [2].

Antioxidant is a molecule that counteracting the toxic effect of free radicals by inhibiting the oxidative chain reaction. Fruit and vegetables are potential source of exogenous antioxidant. Some studies stated that carrot [3], tomato [4], namnam [5], doum fruit [6], noni (*Morinda Citrifolia*) [7], orange [8], blueberry [9], and guava have potent antioxidant activity [10].

Guava has potential antioxidant compounds such as phenolic and flavonoid compounds, ascorbic acid, carotenoids, and lycopene [10]–[12]. Solvent and extraction technique influence the bioactive compounds extracted. Ethyl acetate can extract polyphenols, flavonoids, and tannin as potential antioxidant compounds from walnut green husk [13]. Some studies exhibited the antioxidant activity of ethyl acetate extract from plants and mushroom [14]–[17].

Various extraction method have been used for extracting antioxidant. Ultrasonic extraction is one of the nonconventional methods that are energy-efficient and economically sustainable [2]. Reference [18] proved the ultrasonic extraction is the best technique to extract antioxidant from guava. Therefore, in this study we used

sonicator as an ultrasonic extractor to extract bioactive compounds from guava fruit.

In this research, we determine the antioxidant activity of ethyl acetate extract of guava fruit. We also investigate the phytochemical properties of the extracts to predict the bioactive compounds. Phytochemical screening test were identified by the precipitate formation or the color change.

II. METHODOLOGY

A. Chemicals

DPPH (2,2-diphenyl-1-picrylhydrazyl) was purchased from Sigma-Aldrich and Vitamin C (IPI 50 mg) was from PT. Supra Ferbindo Farma. Methanol p.a., Ethyl acetate p.a., and hexane p.a. were purchased from Smartlab.

B. Plant Material

Pink flesh guava fruit (*Psidium guajava*, Linn) was obtained from the traditional market in Purwokerto, Central Java, Indonesia.

C. Preparation of Extract

The pink flesh guava fruits were washed and crushed to produce uniform slurry without peeling the skin. The slurry sample were extracted using sonicator with ethyl acetate at room temperature. The extraction process was carried out for one hour. The cloudy extracts were centrifuge for 10 minutes at 3,000 rpm. Then, using vacuum rotary evaporator the clear supernatant was concentrated at 50-65 °C.

D. Phytochemical Screening

The phytochemical screening was carried out to identify flavonoids, saponins, tannins, triterpenoids and steroids content. The presence of these metabolites was identified by the precipitate formation and the color change or intensity.

E. Antioxidant Activity

Briefly, 0.1 mM DPPH solution was prepared in methanol solvent. About 2 mL of DPPH solution was mixed with 2 mL of methanolic guava extract solution (0.01-0.13 mg/mL). The mixture was incubated at room temperature and placed in a dark room. After 30

minutes, the antioxidant activity was determined by measuring the absorbance level using UV-Vis spectrophotometer at 515.5 nm. The absorbance data then put in this equation below to calculate the percent inhibition.

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

Furthermore, the IC₅₀ values were determined to identify the antioxidant activity by plotting the percent inhibition versus inhibitory concentration.

III. FINDINGS AND DISCUSSION

A. Phytochemical Study

Phytochemical analysis of ethyl acetate extract of guava fruit are showed the presence of flavonoids, steroids, and tannins. Flavonoids were confirmed using magnesium powder and hydrochloric acid. The formation of reddishblack solution indicated the positive reaction. Terpenoids and steroids identification were conducted using LiebermanBurchard reagent. A blue or green color formation in solution expressed the presence of steroids. Tannins were verified by ferric chloride test. A green, purple, blue or black color developed indicating the presence of tannins.

TABLE I. PHYTOCHEMICAL ANALYSIS

Phytochemicals	Result
Flavonoids	+
Saponins	-
Tannins	+
Triterpenoids	-
Steroids	+

B. Antioxidant Activity

In this research, DPPH method was used to determine the antioxidant activity of guava fruit extract by measuring the fruit extract abilities to donate hydrogen atom to the DPPH radical. The more hydrogen atom donated to DPPH radical, the greater the color change of DPPH radical solution, from purple to yellow color [19]. The absorbance of DPPH solution was monitored by UV-Vis

Spectrophotometer. Then, the absorbance was used to calculate the percent inhibition.

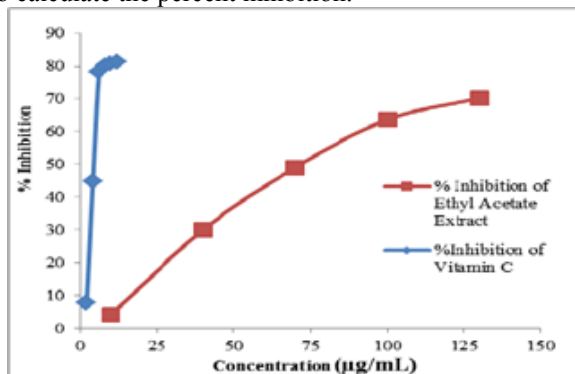


Fig. 1. Percent Inhibition of Vitamin C and Ethyl Acetate Extract of Guava Fruit

Picture 1 showed that the extract concentration is proportional to the percent inhibition. It means, the greater the extract concentration, the more antioxidant in the extract that can reduce free radical activity [20]. Vitamin C as a reference standard has the same inhibition pattern with guava extract. The percent inhibition of Vitamin C was higher than ethyl acetate extract. Based on the linear regression calculation, the IC₅₀ of ethyl acetate extract of guava fruit was 81.92 µg/mL.

Antioxidant activity of guava fruit is higher than other fruits, such as banana, carrot, pineapple, pawpaw (papaya) tomato, watermelon, and orange with the IC₅₀ were 181.86 µg/mL, 228.05 µg/mL, 311.81 µg/mL, 251.51 µg/mL, 277.43 µg/mL, 217.56 µg/mL, and 187.30 µg/mL, respectively [21]. Furthermore, [22] reported that antioxidant activity of watermelon, papaya, pear, and jackfruit are lower than guava.

The antioxidant activity of ethyl acetate extract of guava fruit may be due to the presence of phytochemicals i.e. flavonoids, steroids, and tannins. Some studies have shown that flavonoids and tannins are the potential antioxidant compounds. The high flavonoids contents in the extract exhibited a high antioxidant activity [23], [24]. Furthermore, tannins content in *P. amarusare* enhanced the antioxidant activity [25].

IV. CONCLUSION

Guava fruit can be used as an alternative source of antioxidant. Ethyl acetate extract of guava fruit showed a high antioxidant activity. This extract contains some phytochemicals such as flavonoids, steroids, and tannins. These components are predicted as a potential antioxidant compounds.

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