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The Correlation of Total Phenol Content with Free Radicals Scavenging Activity and Effect of Ethanol Concentration in Extraction Process of Mangosteen Rind (*Garcinia mangostana*)

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***Abstract*—**The use of synthetic antioxidants often causes a negative effect on health and increases the incidence of carcinogenesis. Development of the natural antioxidants should be investigated. However, natural antioxidants have a low toxicity and are safe for human consumption. Ethanol extract of mangosteen rind (*Garcinia mangostana*) contains natural antioxidant compounds that have various pharmacological activities. Antioxidants from the ethanol extract of mangosteen rind have free radicals scavenging activities. The scavenging activity of ethanol extract of mangosteen rind was determined by DPPH method. The phenolic compound from the ethanol extract of mangosteen rind is determined with Folin-Ciocalteu method. The results showed that the absolute ethanol extract of mangosteen rind has IC_{50} of 40.072 ug/mL. The correlation of total phenols content with free radical scavenging activity has an equation y: 5.207x + 205.51 and determination value (R^2) of 0.9329. Total phenols content from the ethanol extract of mangosteen rind has a good correlation with free radicals scavenging activity of DPPH.

***Keyword*—**Antioxidant, *Garcinia mangostana*, inhibition concentration 50%, total phenolic compounds.

I. Introduction

MANGOSTEEN fruit is regarded as the source of the antioxidant compound in the world [1]. The phytochemical screening showed that the ethanol extract of mangosteen rind has active compounds such as alkaloids, flavonoids, glycoside, saponins, tannins, and steroids or triterpenoids [2]. The unique active compound from mangosteen rind is xanthone. Other active compounds from mangosteen rind extract are anthocyanin [3], flavonoids, and tannins [4]-[6]. The active compound from mangosteen rind extract, particularly α-mangostin has antiproliferative effect, suppresses of tumor growth and breast cancer metastasis on mice [7]. Reference [8] also reported that the mangosteen rind extract is able to improve the histology pancreatic of rat induced by streptozotocin (STZ).

Many researches showed that the mangosteen rind (*Garcinia mangostana* L.) contains the active compounds that have pharmacological activity and antioxidant properties. The active compounds from mangosteen rind extract which have beneficial for health [9]. Reference [10] reported that α-mangosteen has the advantage as chemopreventive and/or useful for a complementary alternative medicine in the breast cancer treatment. It is also reported by [6], that the *E. Garcinia* has the ability for cancer treatment. Reference [11] reported that the xanthone derivative like Patuloside A has antibacterial activity. Meanwhile, according to [12], the simple xanthone derivatives also have high antioxidant activity against DPPH free radicals. Reference [13] reported that the antioxidant activity the extract of mangosteen rind has 50% inhibitory concentration (IC_{50}) of 5.94 ug/mL.

Mangosteen rind extract can decrease the blood glucose levels. Reference [2] reported that the ethanol extract of mangosteen rind has antidiabetic effects in mice. Reference [14] reported that the extract of mangosteen rind with a dose of 250 mg / kg and 500 mg / kg can decrease blood glucose levels at 105.92 mg / dl and 134.25 mg /dl for 4 weeks.

The results from previous studies showed that mangosteen rind extract has a wide benefit. Therefore, this research will determine the correlation between total phenol content and free radical scavenging activity of DPPH. The purpose of this study was to know the correlation between total phenol content in mangosteen rind extract and antioxidant activity.

II. Research Methods

A. Materials

The raw material was used the mangosteen rind (bought in fruit market Yogyakarta), ethanol as an extraction solvent. Other chemicals were distilled water, Na_2CO_3 7.5%, DPPH 1 mM Solution, Folin Ciocalteu Reagent.

B. Research Procedure

1. Sample Preparation

Mangosteen rind was dried in the open air for 7 days, and then powdered using a blender.

2. Mangosteen Extraction Process

100 grams of mangosteen rind powder were macerated with 400 ml of absolute ethanol for 24 hours with stirring. The variations the ethanol concentrations are 80%, 50%, 20% and water in the same treatment. After maceration for 24 hours, the solution was filtered using a vacuum and separate the pulp and filtrate. Then the filtrates were evaporated with a vacuum evaporator and then were dried to obtain the dry extract.

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3. Identification of Total Phenol Content

The total phenol was determined using Folin Ciocalteu method. Gallic acid standards were made in various concentrations; 15, 20, 25, 30, 40, 50 and 60 ug/ml. Then, 0.3 mL solution of gallic acid standard was mixed with 1.5 mL solution of Folin Ciocalteu reagent (1:10, v/v) and the solution was stirred until homogeneous. After 5 minutes, 1.2 mL of 7.5% sodium carbonate was added and awaited for 60 minutes. Then the solution was measured at a wavelength of 748.6 nm using UV-Vis spectrophotometer. 100.0 mg sample of ethanol extract of mangosteen rind was diluted with distilled water to a volume of 25.0 ml. Then, the identification of total phenol content of ethanol extract of mangosteen rind was carried out in the same way with procedure of the solution of Gallic acid standard solution.

4. DPPH Activity Assay

Determination of the free radical scavenging activity of mangosteen rind was done by DPPH method. 1.0 ml sample was added 1.0 mL solution of DPPH 1 mM then shaken and awaited for 30 min. The color of the DPPH solution changed from purple to yellow which indicated the free radicals scavenging activity of DPPH. Furthermore, absorbance was measured at 517 nm using a UV-Vis spectrophotometer. The equation of DPPH radical scavenging activity is as:

$$IC_{50}(\%) = \frac{(\text{absorbance of control} - \text{absorbance of sample}) \times 100\%}{\text{absorbance of control}} \quad (1)$$

III. Result and Discussion

A. Characterization of Ethanol Extract of Mangosteen Rind Using FTIR

The extraction process of herbal plants is very important because it will determine the amount of active compound that will be extracted from herbal plants source. Therefore, the extraction process needs to be optimized to obtain the active compound. So, this research will take up the effect of ethanol concentration in the extraction process of active compound from mangosteen rind.

The result of FTIR spectrum showed that there was difference of the functional group vibration from ethanol extract of mangosteen rind. It indicates that the amount of active compounds from mangosteen rind which was extracted have different (Fig. 1).

The vibration of functional groups from absolute ethanol extract of mangosteen rind showed the aromatic alkene vibration in the wavelength of 1500 cm^{-1} and 1600 cm^{-1} with strong intensity. The water extract of mangosteen rind has low intensity in this field. These differences were the result of differences from the type and content of active compounds which were extracted from mangosteen rind. This is evidence that the different of ethanol concentration has different effect and capability in the extraction process.

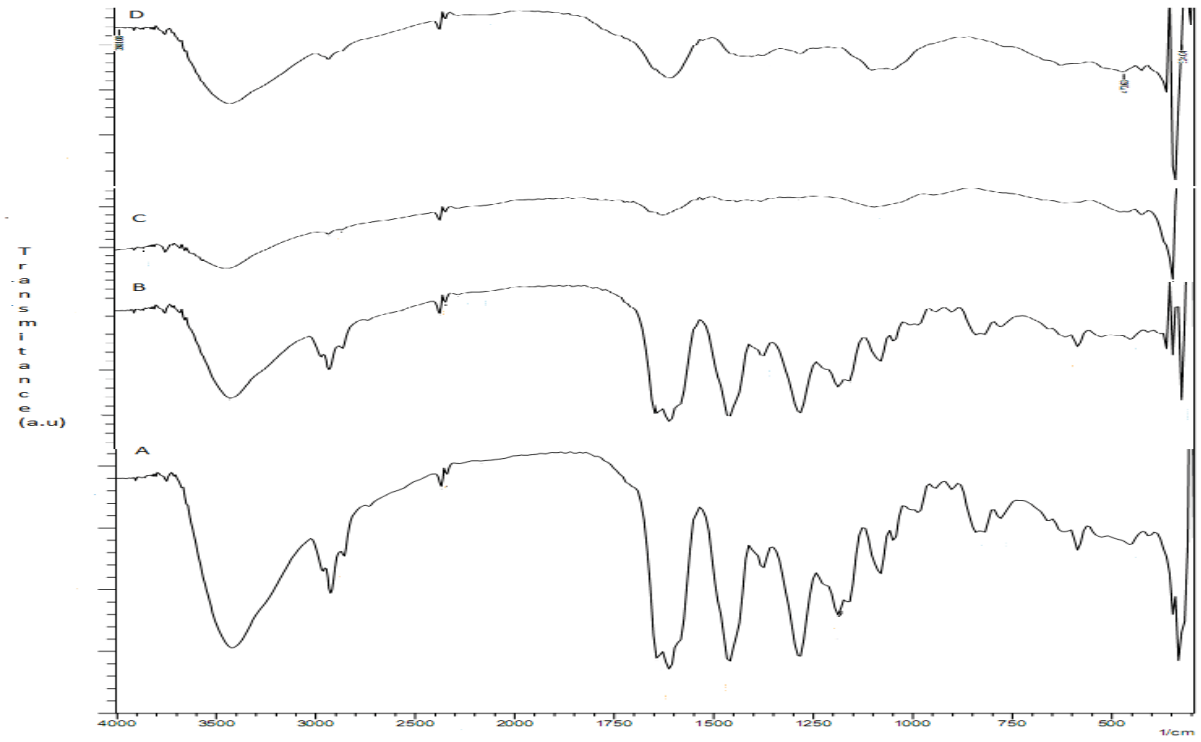

Fig. 1 FTIR spectra of ethanol extract of mangosteen rind with; (A) Absolute ethanol; (B) 80% Ethanol; (C) 50% Ethanol; and (D) water

B. Free Radical Scavenging Activity of DPPH

Free radicals scavenging activity of DPPH from the ethanol extract of mangosteen was stable over 30 minutes. The scavenging activity of DPPH was done at maximum wavelength, 517 nm. The scavenging activity was indicated to change the DPPH color from purple to yellow color.

The antioxidant activity was illustrated by [15] that the free radicals scavenging activity of DPPH depends on the donor ability of proton from the active compound. Therefore, the different of active compound in the ethanol extract of mangosteen rind can provide the different of antioxidant activity. This research showed that increasing the ethanol concentration in the extraction process will get the increasing of the antioxidant activity. The result of this research showed that the absolute ethanol as a good solvent in the extraction process and absolute ethanol extract of mangosteen rind has highest of antioxidant activity from water extract of mangosteen rind. The value of antioxidant activity from the absolute ethanol extract of mangosteen rind is 40.072 ug/mL (Table I).

TABLE I
THE RESULT OF FREE RADICAL SCAVENGING ACTIVITY (IC_{50}) FROM ETHANOL EXTRACT OF MANGOSTEEN RIND

Ethanol concentration	IC_{50} Value (ug/mL)				
	Replication			Average	SD
	I	II	III		
Absolute ethanol	38.889	40.742	40.586	40.072	1.028
80% Ethanol	68.376	68.578	69.431	68.795	0.560
50% Ethanol	182.176	183.548	179.979	181.901	1.800
20% Ethanol	191.321	188.887	191.179	190.462	1.365
Water	217.240	214.093	214.490	215.274	1.714

C. The Correlation of Total Phenol and Free Radical Scavenging Activity of DPPH

Ethanol extract of mangosteen rind has free radicals scavenging activity of DPPH. This is a potential for mangosteen rind to be herbal products. However, the scavenging activity of ethanol extract of mangosteen rind depending on the active compound in the extract. While, the active compound or secondary metabolites in mangosteen rind also depends on composition of soil nutrient where the mangosteen tree was planted. Due to differences in the composition of the active compounds from mangosteen rind tend to design the correlation between the active compound content and antioxidant activity. This study makes a correlation between total phenol content of the mangosteen rind to free radicals scavenging activity, Fig. 2. The result showed that the correlation of total phenol compound toward free radicals scavenging activity of DPPH has a good correlation. The linear line was obtained at an R^2 value of 0.9329.

IV. CONCLUSION

The absolute ethanol is a good solvent in the extraction process of mangosteen rind because of the extract of mangosteen rind has highest antioxidant activity with IC_{50} is 40.072 ug/mL. The correlation of total phenols content toward the free radical scavenging activity of DPPH has a good correlation with R^2 value is 0.9329 and the equation y: 5.207x + 205.51.

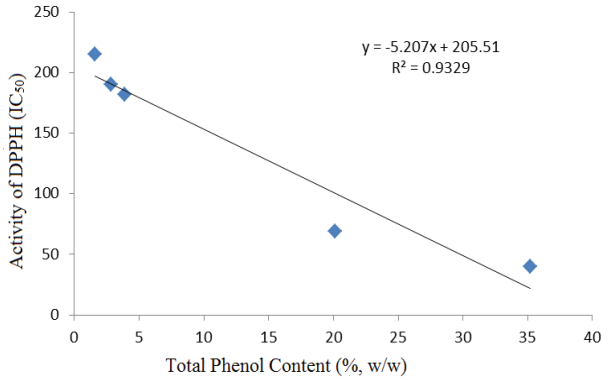

Fig. 2 Correlation of total phenol content and free radical scavenging activity of DPPH

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