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Determination of Total Phenolic Content and Antioxidant Activities from Extract of the Leaf, Fruit Skin and Stem Bark of *Garcinia cowa* Roxb

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Abstract

Leaves and fruits of *Garcinia cowa* Roxb have been used as spices in Indonesia especially in Minangkabau tribes. It is also used in traditional folk medicine for indigestion, improvement of blood circulation, and an expectorant. *G. cowa* Roxb contains phenolic compounds which had activity as antioxidant. The purpose of this research is to know the total phenolic content of ethanol extract of the leaf, rind, and stem bark of *G. cowa* Roxb by using Folin-Ciocalteu assay and to know its antioxidant activities by using FRAP (Ferric Reducing Antioxidant Power) assay. The result of the research found that the total phenolic content of ethanol extract of the leaves, rinds, and stem bark of *G. cowa* Roxb. were 17.172, 9.263 and 20.040 mg/100 gram, respectively, and its antioxidant activities were 25.968, 25.137 and 26.837 mmol Fe(II)/100 gram, respectively, and antioxidant activities of Gallic acid which was used as a reference standard was 9.338 mmol Fe(II)/100 gram.

Keywords: *Garcinia cowa* Roxb., phenolic, antioxidant, Folin-Ciocalteu assay, FRAP assay

1. Introduction

Free radical is a result or product of pollution such as cigarette smoke, radiation, pesticide, vehicle emission, chemical compounds, and poisonous food or fast food (Setiati, 2003). Free radical is reactive and very dangerous for health. Reactivity impact of free radical compounds are damage of cell or tissue, autoimmune disease, degenerative disease, and cancer (Sadikin, 2001).

The conformation of free radical compound can be prevented by antioxidant. Antioxidant can inhibit the reaction of oxidation by binding the free radical and reactive compounds. As a result, cell damage will be inhibited (Winarsi, 2007). One of the plants which has antioxidant activity and has many phenolic compounds is genus Garcinia. Garcinia is one of Guttiferae family which has various numbers of types (Rukmana, 2007). In Indonesia, *Garcinia cowa* Roxb is used as spices especially in West Sumatra. The leaves of *Garcinia cowa* Roxb contain oxygenated and prenylated xanthone, flavonoid, and benzophenone (Ampofo & Waterman, 1986). The stem bark of *Garcinia cowa* Roxb contains *α*-mangostin, cowanin, cowanol, cowaxanthone, rubraxanthone, *β*-mangostin, and tetraprenyltolouquinone (Likhitwitayawuid *et al.*, 1997; Wahyuni *et al.*, 2004). The stem bark has been reported contains cambogin (Jena *et al*., 2002).

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Based on a literature study of the chemical compounds of leaves, rinds, and bark of *G. cowa* Roxb, the phenolic content and antioxidant activity of parts of this plant has never been determined. Therefore, this study aims to determine the total content of phenol compounds and antioxidant power of each part of *G. cowa* Roxb plant. Antioxidant activity was examined using FRAP (Ferric Reducing Antioxidant Power) assay, while total phenolic content was calculated using Folin-Ciocalteu assay.

2. Materials and Methods

2.1 Chemicals and reagents

The chemicals used in this study were ethanol (Merck), Gallic acid (Sigma®), Folin-Ciocalteu reagent (Merck), sodium carbonate (Sigma®), ortho-phenantroline (Sigma®), ferric chloride hexahydrate (Sigma®), distilled water (Brataco®), ferrous sulfate heptahydrate (Sigma®), and sodium acetate trihydrate (Sigma®).

2.2 Plant samples and extraction

Leaves and stem bark of *Garcinia cowa* Roxb were collected from Limau Manih, Padang, West Sumatera, while its rind were collected from Duri, Riau. The plants of samples were identified by taxonomist from Herbarium ANDA, Andalas University.

The leaves, rinds, and stem bark were dried and powdered. The weight of each powder of the leaves, rinds, and stem bark of *Garcinia cowa* Roxb used were 1 kg, 500 gram, and 1 kg, respectively. Leaves and stem bark were divided into five (5) parts. Then each of them is put on the containers. 2 L of 70% ethanol is put into each container. 500 gram of dried powdered sample of the rinds is divided into three (3) parts. Each part is put on the containers. Each container were added 2, 2, 1 L of 70% ethanol, respectively. Maceration is conducted during the first six hours and at the same time it is stirring occasionally, then, kept it motionless about 18 hours. Macerate is filtered by using filter paper. Macerate is combined and concentrated by rotary evaporator to obtain a thick extract (Departemen Kesehatan Republik Indonesia, 2008).

2.3 Characterization of dried powder sample and extract

The checking of organoleptic of the dried powder sample and extract is conducted by organoleptic observation including shape, color, and odor of the extract. The determination of ash content of the extract is conducted by weighing thoroughly 2 gram of samples, and then put it in ceramic container (silicate crust) that has been glowed and tare, glow it till the charcoal is over, and then let in the room temperature before it is weighed. Determination of moisture content of extract was done by distillated the mixture of extract and toluene. Distillation was done by using distillation flask. The water volume was measured since water and toluene were perfectly splinted. Moisture content was calculated in % v/w (Indonesia, 2008).

2.4 Preparation of solutions

2.4.1 Preparation of test solutions to determine the total phenolic content

Weighing thoroughly 5.1 mg of extract and dissolved by 10 ml of ethanol.

2.4.2 Preparation of test solutions to determine the antioxidant activities

Weighing thoroughly 12.5 mg of extract and dissolved by 10 ml of ethanol.

2.5 Determination of maximum absorption wavelength

2.5.1 Determination of maximum absorption wavelength of gallic acid − Folin-Ciocalteu

An amount of 0.1 mL of gallic acid as the comparison solution is put together with 0.5 mL of Folin-Ciocalteu reagent by using pipette with the concentration 200 µg/mL then, added it into 0.9 mL of distilled water. Then, the mixture is being the vortex and kept it motionless about 5 minutes. Then, the mixture is added by 2.5 mL of 7% sodium carbonate solution, then, it becomes a vortex. Keep it motionless in the room temperature for about 26 minutes (Mustafa *et al.*, 2010). After that, determine the maximum absorption wavelength by using UV-Vis spectrophotometer in 400-800 nm of range.

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2.5.2 Determination of maximum absorption wavelength of 10 mmol/L ferrous sulfate heptahydrate solution

An amount of 5 mL of acetate buffer 0.3 M (pH 3.6) is put into the test tube by using pipette. Then, add 0.5 mL of 0.5 mmol/L of ferrous sulfate heptahydrate standard solution and 0.5 mL of 10 mmol ortho-phenantroline solution. Keep it motionless for 30 minutes in dark place, then determined the maximum absorption wavelength by using UV-Vis spectrophotometer in 400-800 nm of range (Harris, 2007).

2.6 Linearity

2.6.1 Gallic acid − Folin-Ciocalteu

By using pipette 0.1 mL of a standard solution of gallic acid in five different concentrations (i.e. 100 µg/mL, 150 µg/mL, 200 µg/mL, 250 µg/mL, and 300 µg/mL) and 0.5 mL of Folin-Ciocalteu's reagent, added to 0.9 mL of distilled water. The mixture was then agitated by vortex and keeps it motionless for 5 minutes. The mixture is then added 2.5 mL of 7% sodium carbonate solution and then agitated by vortex. Keep it motionless for 26 minutes at room temperature (Mustafa *et al.*, 2010). Then the absorbance was measured at the maximum absorption wavelength of 764.5 nm, and then created a calibration curve by plotting the absorbance obtained from analysis of a standard concentration in order to obtain a linear regression line equation (y = a + b x). Linearity is determined by the value of r (regression coefficient).

2.6.2 Solution of 10 mmol/L ferrous sulfate heptahydrate

By using pipette each of 0.5 mL of standard solution of ferrous sulfate heptahydrate in five different concentration (0.3 mmol/L; 0.4 mmol/L; 0.5 mmol/L; 0.6 mmol/L; and 0.7 mmol/L), then added 0.5 mL of ortho-phenantroline and then put in a test tube which had contained 5 mL of 0.3 M acetate buffer (pH 3.6). Also prepare blank solution without a standard solution containing ferrous sulfate heptahydrate. The solution is left for 30 minutes in a dark place and recorded absorbance at the maximum absorption wavelength of 509.6 nm (Harris, 2007). A calibration curve was made by plotting the absorbance obtained from the analysis of the standard concentration thus obtained linear regression equation (y = a + bx). Linearity is determined by the value of *r* (regression coefficient).

2.7 Sensitivity

Sensitivity is determined by calculating the value of limit of detection (LOD) and limit of quantization (LOQ).

$$LOD = \frac{3 \times SD}{b}$$

$$LOQ = \frac{10 \times SD}{b}$$

Note: LOD = limit of detection
LOQ = limit of quantization
SD = standard deviation of the residuals
b = slope of regression equation

2.8 Determination of total phenolic content by using Folin-Ciocalteau assay

Determination of total phenolic content is conducted by using pipette in which 0.1 mL and 0.5 mL of Folin-Ciocalteu reagent solution is added to 0.9 mL of distilled water. Then, the mixture was being a vortex and keeps it motionless for 5 minutes. The mixture is then added 2.5 mL of 7% sodium carbonate solution and then vortex. Keep it motionless for 26 minutes at room temperature (Mustafa *et al.*, 2010). The absorbance was measured at the maximum absorption wavelength of 764.5 nm.

2.9 Determination of antioxidant activity using Ferric Reducing Antioxidant Power (FRAP) assay

A total of 0.1 mL of the test solution and 0.3 ml of distilled water added to the tubes already containing 3 mL of FRAP reagent, and then the mixture is being a vortex and incubated at 37 ^{0}C for 30 minutes in a dark place at room temperature. Sample absorbance is measured at the maximum absorption wavelength of 509.6 nm. FRAP reagent solution with distilled water without samples were used as a reference solution. The antioxidant

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activity of the sample is shows in the ferrous equivalent (Fe^{+2} mM) using a standard curve of 0.3, 0.4, 0.5, 0.6, and 0.7 mmol/L ferrous sulfate heptahydrate.

3. Results and Discussion

The extraction method used is maceration with ethanol because it is an easy method and uses a simple tool (Rivai *et al*., 2008; Martinus & Rivai, 2015). All filtrate obtained from the extraction was evaporated using a rotary evaporator to obtain a thickened extract of leaf, fruit skin, and bark of 115.4, 259.2 and 308.7 gram, respectively. The yield obtained from each sample was 11.5, 51.8, and 30.9%. The qualitative test of phenolic compound with 1% iron (III) chloride solution in the extract showed positive result. Characterization of sample was done by measuring ash content and water content. The characterization results are shown in Table 1.

Table 1: Characteristics of samples

Samples	Ash content	Water content
Leaves	7.402%	7.996%
Fruit skin	3.151%	10.624%
Stem Bark	0.98%	12.193%

In the determination of the maximum absorption wavelength, a solution of gallic acid with a concentration of 200 µg / mL is used. The UV-Vis spectrophotometric reading results at a wavelength of 400-800 nm showed an absorption of 0.581 at a maximum absorption wavelength of 764.5 nm (Figure 1). The maximum absorption wavelength is slightly different from the literature, i.e. 765 nm (Prior *et al*., 2005). This is due to the difference in instrument type and purity of the gallic acid used. However, this value still falls within the tolerance limit of the tool used, which is ± 2 nm.

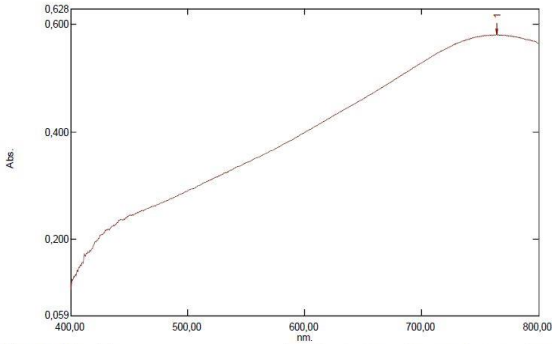

Figure 1: The maximum absorption spectra of gallic acid with the Folin-Ciocalteu at a concentration 200 µg/mL in distilled water

In the determination of linearity, the measurement of absorbance of gallic acid was performed at concentrations of 100 µg / mL, 150 µg / mL, 200 µg / mL, 250 µg / mL, and 300 µg / mL, respectively. This measurement yields calibration curve with regression equation y = 0.00206 x + 0.13057 and correlation coefficient value r = 0.99973 (Figure 2). The absorbance measurement of these gallic acids is useful in determining the total phenolic concentration of the sample by using the regression equation of the gallic acid calibration curve with the Folin-Ciocalteu reagent. A value of r close to 1 proves that the regression equation is linear (Rohman, 2014).

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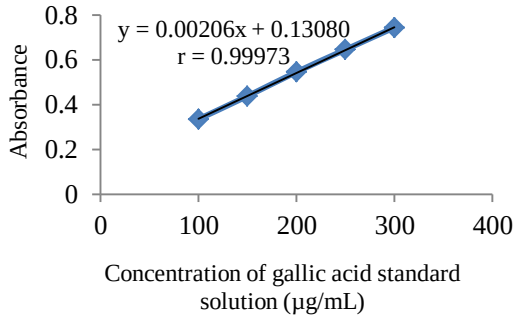

Figure 2: Calibration curve of gallic acid with Folin-Ciocalteu reagent

The limits of detection (LOD) in the standard solution of gallic acid showed a value of 4.368 µg / mL, meaning that the value is the smallest concentration of the analyte that can be measured by UV-Vis spectrophotometry. The limit of quantitation (LOQ) in the standard solution of gallic acid showed a value of 14.563 µg / mL. This number indicates that the lowest gallic acid concentration in the sample solution is in the determination of precision and accuracy acceptable by the Folin-Ciocalteu method at a good level of accuracy and accuracy.

The test solution for determining the total phenolic content of the extract was prepared with a certain concentration whose absorbance was in the linear line of the calibration curve, i.e. at a concentration of 1000 µg / mL. In the determination of total phenolic content of ethanol extract of *G. cowa* Roxb, it was found that total phenolic content of leaf extract, fruit skin, and bark of *G. cowa* Roxb were 17.172, 9.263, and 20.040 mg / 100 g, respectively.

The method used for the determination of antioxidant activity is the Ferric Reducing Antioxidant Power (FRAP) method. Acetate buffer is used to stabilize the pH of the solution. The test is carried out at acidic pH because iron dissolves in acidic atmosphere and prevents the formation of hydroxide precipitate occurring at an alkaline atmosphere. The results obtained in determining the maximum absorption wavelength for this method is 509.6 nm (Figure 3). The maximum absorption wavelength is close to the maximum absorption wavelength present in the literature, i.e. 510 nm (Cools *et al*., 2011). The calibration curves in this method were made at concentrations of 0.3 mmol / L, 0.4 mmol / L, 0.5 mmol / L, 0.6 mmol / L, and 0.7 mmol / L, respectively. The resulting absorbents of each of these concentrations were 0.257, 0.351, 0.444, 0.539, and 0.635, respectively. The calibration curve obtained shows the regression equation y = 0.94531x – 0.02734 and the value r = 0.99997. This implies that this result is linear (Figure 4). According to the literature the relation coefficient (r) shows a linear result when the value of r is close to 1 (Gandjar & Rohman, 2007).

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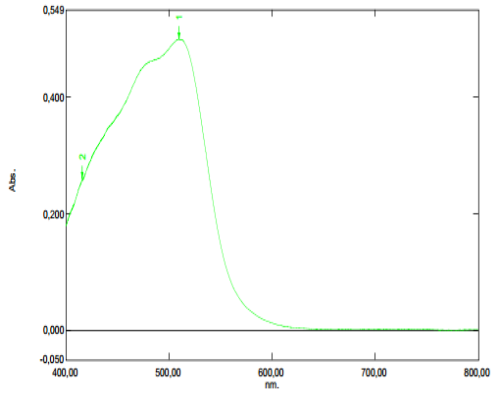

Figure 3: The absorption spectrum of a standard solution of ferrous sulfate heptahydrate and ortho-phenantroline

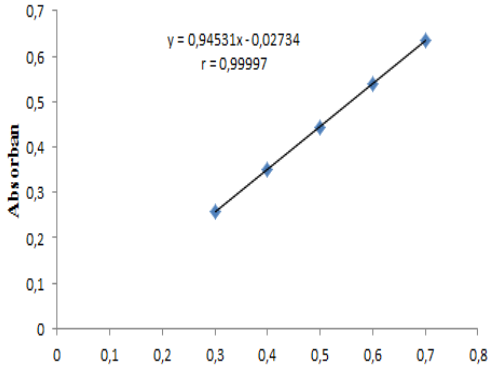

Figure 4: The calibration curve of standard solution of ferrous sulfate heptahydrate and ortho-phenantroline

Sensitivity is determined from the calculation of the detection limit value (LOD) and the quantitative limit (LOQ). The values of LOD and LOQ obtained were 0.003 mmol / L and 0.011 mmol / L, respectively. Determination of antioxidant activity showed that antioxidant activity of ethanol extract of *Garcinia cowa* Roxb stem bark was 26.837 mmol Fe (II) / 100 g, fruit skin 25.137 mmol Fe (II) / 100 g, and leaves 25.968 mmol Fe (II) / 100 g. While gallic acid as a comparator showed antioxidant activity of 9.338 mmol Fe (II) / 100 g.

4. Conclusion

Based on the research that has been conducted it can be concluded as follows: Total phenolic content of ethanol extract of the leaves of *Garcinia cowa* Roxb is 17.172 mg/100 g, the fruit skin is 9.263 mg/100 g, and the stem bark is 20.040 mg/100 g.

The antioxidant activity of ethanol extract of the leaves of *Garcinia cowa* Roxb is 25.968 mmol Fe (II)/100 g, the fruit skin is 25.137 mmol Fe (II)/100 g, and the stem bark is 26.837 mmol Fe (II)/100 g. The antioxidant activity of gallic acid as the reference standard is 9.339 mmol Fe (II)/100 g.

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