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Total Phenolic Content of Cortex and Leaves Of Ramania (*Bouea macrophylla* Griffith) And Antioxidant Activity Assay by DPPH Method.

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ABSTRACT

Phenol compounds are phytonutrients that have an antioxidant properties. Antioxidant are substances that the body needs to neutralize free radicals and prevents the damage caused by free radicals on normal cells, proteins, and fats. Ramania or Gandaria (*Bouea macrophylla* Griffith) is a species of Anacardiaceae, was reported that the fruit have an antioxidant activity. This plant is found in several different areas in Indonesia with a variety of different names, but it is still have a very limited utilization. In South Kalimantan, Ramania can be eaten as salad, pickles, and used as a substitute for lemon juice or tamarind. This research was conducted to determine the total phenolic content and the antioxidant activity of cortex and leaves of Ramania. Cortex and leaves of Ramania were macerated using ethanol and evaporated to obtain the extract ethanol of Ramania cortex and extract ethanol of Ramania leaves. Qualitative test using $FeCl_3$ 1% indicates that the ethanol extract of cortex and leaves of Ramania were positive contain phenolic compounds. The levels of total phenolic compounds determined using the Folin-Ciocalteu method and gallic acid as standard, while the antioxidant activity determined using DPPH method. Based on the results, total phenolic content in ethanol extract of Ramania cortex was 136.99 ± 0.11 GAE ($mg.g^{-1}$) while total phenolic content in ethanol extract of Ramania leaves was 68.53 ± 1.37 GAE ($mg.g^{-1}$). The results showed that ethanol extract of Ramania cortex has a higher antioxidant activity with IC_{50} value 20.03 µg/mL compared to those of ethanol extract of Ramania leaves (IC_{50} 55.83 µg/mL), respectively.

Keywords: Ramania, total phenol content, antioxidant activity, DPPH

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INTRODUCTION

Fruits and vegetables are holding a small role in the calories intake per day, but they have more benefits for human health. These benefits are due to vitamin and provitamin such as ascorbic acid, tocopherols and carotenoids. in addition, all fruits and vegetables are rich of phenolic compounds[1,2,3]. Phenolic compound is a phytonutrients that have antioxidant properties[4,3]. Phenolic compounds divided into five classes, including phenol, phenolic acid, hydroxamate derivates and flavonoids.

The ability of phenolic compounds as antioxidant have been widely reported by various studies[5,6,7,8,9]. The phenolic compounds are also known to play an important role in inhibiting oxidation process by free radical by inhibiting initiation or propagation process, thus it can prevent oxidative damage in human body. Oxidative damage may significantly increase the risk factor of chronic diseases development such as cancer and cardiovascular diseases [10, 11, 12, 3].

One of the fruits that have not been many studied as medicinal plant is Ramania (Figure 1). Ramania or Gandaria (*Bouea macrophylla* Griffith) is a species of *Anacardiaceae*, which in some areas in Indonesia were called with various different names, derived from Indonesia and Malaysia. This plant still have a very limited utilization. The Ramania's wood is widely used to make agricultural tools, young leaves can used as salad, the fruit can be eaten, made salad, pickles and juices, as well as used as a substitute for lemon juice or tamarind [13, 14]. The lack of utilization was due to the limitted studies of *B. macrophylla.*

Screening of metabolite content in *B. macrophylla* juice have reported by Lolaen *et al.* (2013). Based on their study, *B. macrophylla* juice shown antioxidant activity by IC_{50} value of 36.4 mg/ml [15]. Recent research showed that the leaves and cortex extracts of plant were containing phenolic compounds, flavonoid and can be used as a natural antioxidant [16, 17, 18, 19, 20]. Therefore, this study was conducted to evaluate the antioxidant potency of ethanol extract of *B. macrophylla*'s leaves and cortex by determine of total phenolic content (TPC) and antioxidant activity.

MATERIAL AND METHODS

Plant Materials

Cortexs and leaves of *B. macrophylla* were collected from Cempaka, Banjarbaru, Kalimantan Selatan in August, 2016. All plant material were washed in water, dried in a forced-air oven at temperature below 50°C, and then grounded separately using an electric mill to obtain the fine powder of each cortex and leaves of *B. macrophylla* .

Extract Preparation

Extraction process was carried out as Azwanida [21] method of extraction with slight modification. Approximately 500 g of powdered leaves and 500 g of powdered cortex was placed separately in a clean glass container and soaked in ethanol 96% (1:7) for the 1st day of maceration. The container with its contents was sealed and kept for 24 hours. Subsequently, the entire mixture filtered by Whatman filter paper. The lees of filtration process was subjected for remaceration process using ethanol 96% (1:4) for the second and third day. After filtration, each filtrate was evaporated at 70°C using a vacuum rotary evaporator to obtain the ethanol crude extract.

Chemical Substances

1,1-diphenyl-2-picrylhydrazyl (DPPH) and gallic acid were purchased from E. Merck. sodium carbonate (Na_2CO_3), Folin-Ciocalteu's reagent (10%) from E.Merck, ethanol (96%) and water distillated.

Determination of total phenolic content (TPC)

The level of TPC of crude ethanol extracts was determined spectrophotometrically using Folin-ciocalteu's reagent and gallic acid as a standard as Jain *et a*l. [22] method with slight modification.

Briefly, 1.0 ml of each extract (250 µg/ml), gallic acid were mixed with 5.0 mL of Folin-Ciocalteau's reagent and 4.0 ml Na_2CO_3 (7.5%). The mixture was allowed to incubate in the dark at room temperature for 1 hour and the absorbance was measured using spectrophotometer UV-vis

Antioxidant activity Assay

The extracts were further assessed using the DPPH method described by Jain *et al.*[22] with slight modifications. The extracts solved in ethanol 96% at different concentrations (30; 35; 40; 45; 50 and 55 µg/ml). Next, 1.0 ml of each sample were added with 1.0 ml of 0.2 mM DPPH. The mixture was incubated in the dark at room temperature for 40 minutes. The absorbance was measured by visible spectrophotometer with ethanol as a blank. The percentation of DPPH inhibition calculated by using the equation:

$$\%\ \text{inhibition} = [(\text{Ac} - \text{As})/\text{Ac}] \times 100$$

Whereas, Ac is the absorbance of DPPH and As is the absorbance of sample.

Statistical Analysis

All data of TPC determination and antioxidant activity were analyzed by linear regresion equation.

RESULT AND DISCUSSION

Extract Preparation

Ramania leaves ethanol extract obtained was 105.349 g with percent yield amounted to 21.07%, while the weight of the ethanol extract of Ramania cortex obtainedwas 54.15 g with percent yield amounted to 10.83%.

Determination of Total Phenol Content

In the determination of TPC, gallic acid was used as a standard solution with concentration series of 40, 60, 80, 100 and 120 µg/mL. The standard curve of gallic acid was made as an equivalent comparison of phenolic compounds contained in ethanolic extract of Ramania leaves and cortex, therefore it was used to determine the level of TPC.Each sample and standard solution were reacted with Folin-Ciocalteu reagent and Na_2CO_3 solution, and subsequently incubated for 1 hour. The reduction of the Folin-Ciocalteu reagent by phenolic compounds will cause a color change of Folin-Ciocalteu into blue color [23]. Reductions will be increased if the extract containshigher phenolic compounds, thus will cause the complex forms darker color and increased the absorbance [24]. Absorbance measurements carried out at 765 nm with 3 times of replication. Figure 2 shows the standard curve of gallic acid,the linear regression equation was y = 0.0043x + 0.0833 and R^2 of 0.9926, respectively.

Based on the linear equation obtained, TPC of ethanol extract of Ramania leaves and cortex can be determined. The results can be seen in Table 1.TPC of ethanol extract of Ramania leaves was 68.53±1.37 GAE $mg.g^{-1}$meanwhile the TPC of ethanol extract of Ramania cortex was 136.99±0.11 GAE $mg.g^{-1}$.

Antioxidant activity assay

DPPH method has been widely used to establish the effectiveness of an antioxidant in the inhibition of free radicals. DPPH is a radical compound which can absorb visible light at 517 nm. The color change occurred during the reaction between DPPH and antioxidants thus caused a declineof absorbance at 517 nm (Liang and Kitts, 2014). In this study, the profile of % DPPH inhibition can be seen in Figures 3 and 4. Based on the results of the antioxidant activity assay of Ramania leaves and cortex ethanol extract, % inhibition value of 47.38% was obtained from Ramania leaves ethanol extract at 55 µg/mL, meanwhile Ramania cortex ethanol extract at the same concentration turned out to provide greater value (88.98%). This finding shows that Ramania cortex ethanol extract has the ability to inhibit free radicals better than Ramania leaves ethanol extract.

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A linear equation,y = 0.8094x + 4.8104 with R^2 = 0.9095 was obtained based on the linear relationship between% inhibition and concentrations of Ramania leaves and cortex ethanol extract for Ramania leaves extract, while linear equation y = 1.3755x + 15.574 with R^2 = 0.9790 was obtained for Ramania cortex. IC_{50} was calculated by the linear equations obtained, the result shows that IC_{50} valueof Ramania cortex was better than the ethanol extract of Ramania leaves as shown in Figure 5. Ramania cortex ethanol extract at concentration of 25.03 µg/mL have been able to give 50% inhibition, while the ethanol extract of Ramania leaves required concentration of 55.83 µg/mL to be able to give 50% inhibition.

a.

b.

c.

Figure 1. a. Ramania's Fruit b. Ramania's leaves c. Ramania's cortex

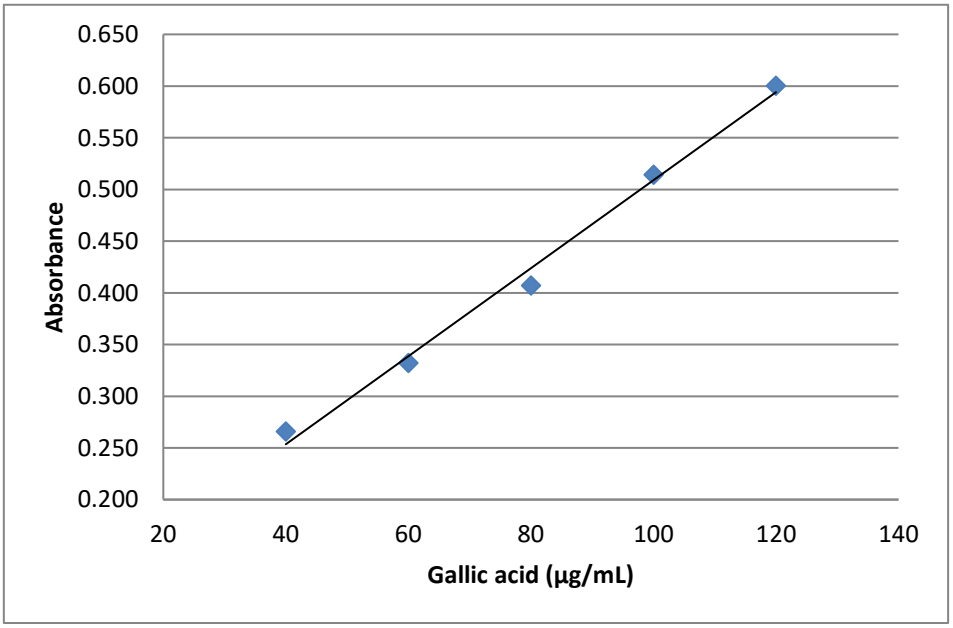

Figure 2. Standard curve of gallic acid

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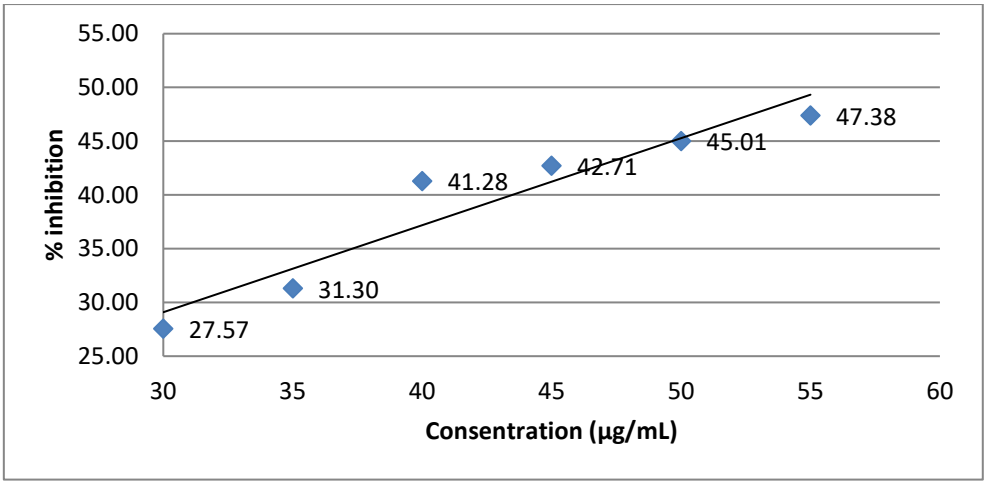

Figure 3. % DPPH inhibition of Ramania leaves ethanol extract

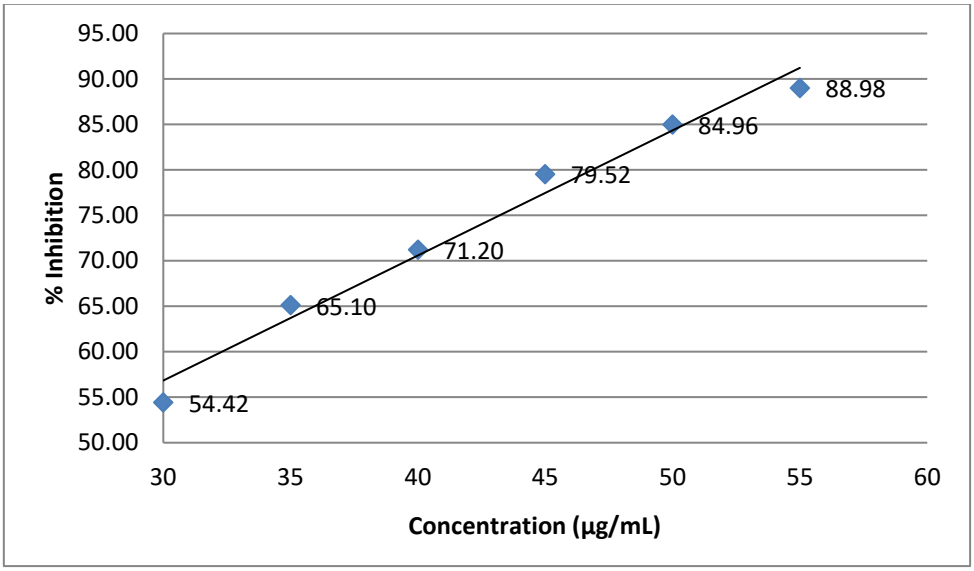

Figure 4. % DPPH inhibition of Ramania cortex ethanol extract

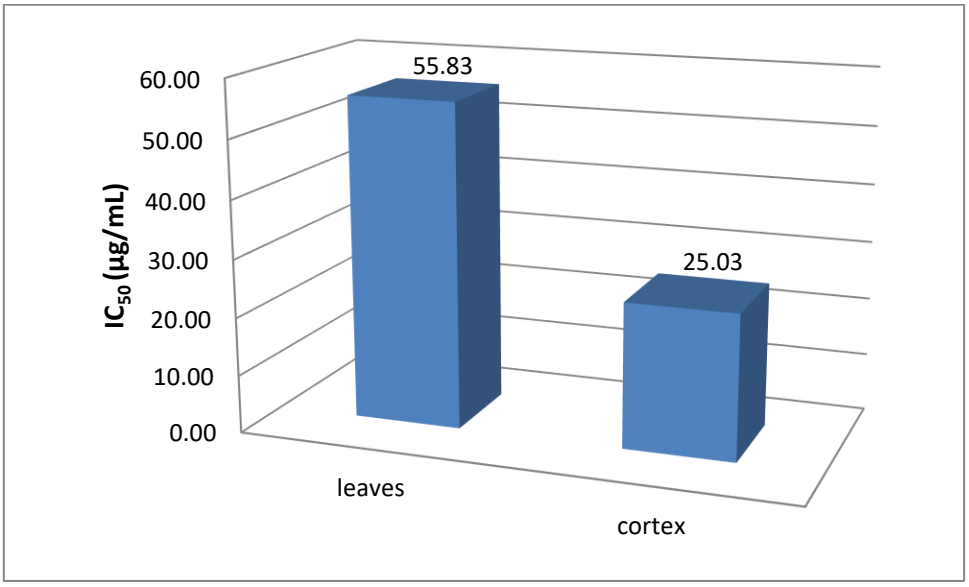

Figure 5. IC_{50} value of Ramania leaves and cortex ethanol extract

CONCLUSION

The results showed that TPC value in ethanol extract of Ramania cortex (136.99 ± 0,11 GAE $mg.g^{-1}$) was greater than TPC value in ethanol extract of Ramania leaves (68.53 ± 1,37 GAE $mg.g^{-1}$). The result of antioxidant activity assay revealed that ethanol extract of Ramania cortex has a higher antioxidant activity with IC_{50} value 20.03 µg/mL compared to those of ethanol extract of Ramania leaves (IC_{50} 55.83 µg/mL). Based on the results, it can be concluded that the higher TPC value of Ramania also gives higher antioxidant activity.

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