



ANTIOXIDANT ASSAY OF THE ETHANOLIC EXTRACT OF THREE SPECIES OF RATTAN FRUITS USING DPPH METHOD

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ABSTRACT

The bioactivity of some species of rattan fruit has been previously studied and it was found that it has potential antioxidant agents. This study was conducted to determine the antioxidant content in three species of edible rattan fruit, namely *Calamus manan* Miq., *Calamus ornatus* Bl. and *Calamus caesius* Bl by using the DPPH method, whereby the test was carried out on the pericarp, flesh, and seeds of each fruit. The research findings revealed that phytochemical compounds, i.e. flavonoids, tannins, and triterpenoids are found almost in all parts of the fruit, while steroid is not found in any of the three species of the fruit. The antioxidant activity found in the fruit of *C. caesius* Bl is strong, meanwhile the antioxidant activity in the fruit of *C. manan* Miq only strong in its pericarp and seeds, but it is moderate in its flesh. The antioxidant activity found in the fruit of *C. ornatus* Bl. is strong in its pericarp and seeds, but it is moderate in its flesh. Therefore, these three species of rattan fruit are potential as antioxidant agents.

Keywords: antioxidant, calamus, flesh, pericarp, seed

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INTRODUCTION

Rattan is a typical tropical plant whose stem is utilized as raw materials to produce a variety of crafts and furniture. Besides its stem, rattan also produces

edible fruit that in several places this fruit can also be useful for health. Ripa [1] stated that the fruit of *Calamus rotang* L was potential for an anti-diarrheal agent. According to [2], rattan fruit can serve as a

medicine to relieve cough, diabetes, and abdominal disorders. Furthermore, he stated that rattan fruit contains Vitamin A and C, and minerals such as Ca, Fe, Mg, Zn and K. Uddin [3] stated that the fruit of *Calamus tenuis* Roxb is used to treat diarrhea.

The three species of fruit analyzed in this study are the species of rattan which commonly grow in the forest area of East Kalimantan. They are *C. manan* Miq., *C. caesius* Bl. and *C. ornatus* Bl., all of which bear edible fruit. Until recently, the fruit of the three species of rattan has not been utilized maximally, except for its stems which have been known as having a high commercial value. The information about the benefits of rattan fruit is not sufficiently available. The knowledge and experiences about its benefits are limited only to the local people living around the forests. They make use of this fruit to relieve pains in a toothache and to treat a diarrhea. This information can be used as a preliminary guide to developing rattan fruit especially with regard to benefits for human health. Some species of rattan fruit that have been studied include *C. erectus* Roxb which contains bio-activity which is potential as antioxidant and antidiabetic agents because of its high content of phenol [4]. Moreover, Ahmed [5] in his study on *C. tenuis* Roxb. found that the phytochemical content in the rattan fruit is related to its bio-activity potentials, that is, antioxidant and cytotoxic.

Based on the background above, this study intended to screen the phytochemical compounds and to determine antioxidant capacity of the three species of rattan fruit, namely *C. manan* Miq., *C. caesius* Bl., and *C. ornatus* Bl by using DPPH method. The parts of the fruit assayed were its pericarp, flesh and seeds. The results of the study will show the part of the fruit that has a better antioxidant content and also will know the type of

rattan which has better antioxidant properties.

MATERIALS AND METHOD

Plant Materials

The fruits of the three species of rattan, i.e. *C. manan* Miq., *C. caesius* Bl., and *C. ornatus* Bl. each separated from the pericarp, flesh and seeds, then each part of the fruit that has been separated dried at room temperature before they were processed to produce rattan fruit powder. Then, the fruit powder was extracted by using maceration method with ethanol as a solvent. The powder sample was soaked in the ethanol for 48 hours with the ratio of 1: 10 (powder: solvent). The result of maceration was evaporated using rotary evaporator at the temperature of 30-40 °C so that rough extract was obtained.

Reagents

Ethanol, ethyl acetate, DPPH (*1,1-diphenyl-2-picrylhydrazyl*), DMSO (dimethyl sulfoxide), distilled water, chloroform, sulfuric acid, hydrochloric acid, acetate lead solution, potassium iodide, acetic acid, sodium hydroxide, alcohol, and ascorbic acid.

Phytochemicals screening

Alkaloids assay [6]

An amount of 1 ml of dragendorff's solution was added into 5 ml of extract and added with 2 ml of HCl. If the color of the solution turned into orange or red color, it indicated that the extract contained alkaloids.

Flavonoids assay [6]

An amount of 1 ml of extract was mixed with some drops of dilute sodium hydroxide (NaOH 1%). An intense yellow colour was appeared in the test tube. It became colourless when on addition of a

few drop of dilute acid that indicated the presence of flavonoids.

Saponin assay [7]

Saponin was assayed by pouring 10 ml of hot water in the reaction tube containing 1 ml of the tested sample which had been dissolved into acetone. Then, the solution was cooled and shaken for 10 seconds. The formation of a steady foam which occurred for 10 minutes with the height of 1-10 cm and would not disappear when it was given 1 drop of HCl 2N, indicated the extract contained saponins.

Tanin Assay [6]

Tannin was assayed by putting 10 ml extract solution into a reaction tube and adding acetate lead solution $(\text{CH}_3\text{COO})_2\text{Pb}$ 1%. Tannins were positive if there was yellow sediment found in the reaction.

Triterpenoid and Steroid Assay [7]

Triterpenoids and Steroids were identified by using the mixture of acetic acid anhydride and concentrated sulfuric acid. This is commonly known as Liebermann-Burchard reaction. Ten drops of acetic acid anhydride and 2 drops of concentrated sulfuric acid were added in series into 1 ml of the tested sample which had been dissolved in acetone. The tested sample was shaken and left for several minutes. Red and purple colors found in the reaction showed the presence of triterpenoids, while green and blue colors indicated the presence of steroids.

Antioxidants Analysis

Antioxidants were analyzed by using a method suggested by [8] with a modification. The absorbance of the extracted sample toward free radicals was assayed by using Spectrophotometer at a room temperature (25 °C) with the wave length of 517 nm and using DPPH (1,1-diphenyl-2-picrylhydrazyl) solution, which

served as free radicals and ascorbic acid was used as a positive control.

Referring to the values of absorbance in the extracted sample, the antioxidant activities were determined based on the percentage of relative inhibition using the following equation:

$$\text{Relative Inhibition Activity (\%)} = \frac{A_0 - A_1}{A_0} \times 100\%$$

where A_0 is the absorbance of DPPH, and A_1 is the absorbance of extract sample. The antioxidant activities were determined based on the value of IC_{50} (inhibitory concentration), i.e. the amount of concentrated solution of sample needed to inhibit 50 % of DPPH, which was calculated by using regression equation where the extract concentration served as its abscissa (axis X) and the value of the relative inhibition served as its ordinate (axis Y). The value of IC_{50} was calculated at 50% relative inhibition using the equation as follows: $Y = b + aX$

RESULT AND DISCUSSION

Phytochemical Contents

Phytochemical is a compound which gives plants their distinctive smells, tastes and colors. The Phytochemical compound also gives a variety of potential benefits for human health. According to [9] some phytochemical components in fruit such as tannin, flavonoids, and triterpenoids serve as inhibitors against free radicals and function as antioxidant agents. In this research, phytochemical testing was carried out on ethanol extracts on pericarp, flesh and seeds of the three species of rattan fruit. Ethanol does not hydrolyze compounds and it is easy to evaporate because of its low boiling point and it is good for thermolabile compounds. [7] reported that the use of ethanol was good for initial extraction. Flavonoids, alkaloids, tannins, saponins, triterpenoids, and

steroids are the phytochemical compounds which were assayed in this research. The assays were conducted to the pericarp,

flesh, and seeds of the three species of rattan fruit.

Table 1. Phytochemical contained in fruit on three species of rattan

Samples	Flavonoid	Alkaloid	Tannin	Saponin	Triterpenoid	Steroid
<i>C. manan</i> Miq.						
Pericarp	+	+	+	+	+	-
Flesh	+	-	+	+	+	-
Seed	+	+	+	-	+	-
<i>C. caesius</i> Bl.						
Pericarp	+	+	-	-	+	-
Flesh	+	-	+	+	+	-
Seed	+	+	+	-	+	-
<i>C. ornatus</i> Bl.						
Pericarp	+	+	+	-	+	-
Flesh	-	-	+	-	+	-
Seed	+	-	+	-	+	-

Note : presence (+)
absence (-)

The results of the assays on Table 1 shows that the fruit extract of *C. manan* Miq contains flavonoids, tannins, and triterpenoids which are found in its pericarp, flesh, and seeds; alkaloids are found in its pericarp and seeds; saponins are found in its pericarp and seeds, while steroids are not detected. The fruit extract of *Calamus caesius* Bl. contains flavonoids and triterpenoids in its pericarp, flesh, and seeds; alkaloids in its pericarp and seeds, tannins in its flesh and seeds; saponins in its flesh; but steroids are not detected. The fruit extract of *Calamus ornatus* Bl. contains flavonoids, tannins, and triterpenoids which are found in the three parts of the fruit; alkaloids are found in its pericarp; but saponins and steroids are not detected. Generally, flavonoids and triterpenoids are identified in the three species of fruit, while steroids are not detected. Alkaloids are commonly found in the pericarp and seeds, but they are not

detected in the flesh of the fruit. Tannins were found almost in all parts of the fruit, except in the pericarp of *C. caesius* Bl. Saponins were not evenly distributed in the three species of fruit. Especially in *C. ornatus* Bl., saponins are not found at all. The findings showed that phytochemical compounds are observed and distributed in three different species of rattan fruit, in which the parts of the fruit were assayed separately. A study conducted by [10] on the *Lepisanthes amoenea* fruit showed a similar result, where flavonoids are found in all parts of the fruit. In addition, the distribution of phytochemical compounds are mostly found in seeds and pericarp. Some previous studies showed that the existence of phytochemical compounds in plants are influenced by some factors such as adaption to the environmental factor and the growth of each individual species. According to [11], the distribution of alkaloid content in various species of plant

is different, depending on the environmental factors and the specific growth of each type of plant. Toxic feature in alkaloid is very useful as the defense of plant against predators. Konarska and Domaciuk [12] stated that tannins are mostly accumulated in the pericarp and mesocarp of *Viburnum opulus* and *Viburnum lantana*. Moreover, according to [13], there are some factors which influence the composition and distribution of saponins in plants, for example, the protection level against predator attacks and the changes of the environment; the protection on the reproductive organs during the growth of fruit and the formation of tubers. The existence of phytochemical compounds in the three species of rattan fruit in this study indicates their potentials as natural antioxidant agents.

Antioxidant Capacity

The antioxidant capacity were assayed by using DPPH method with ascorbic acid as its positive control. The parameter of this method was expressed as the value of the Inhibition Concentration of 50% (IC₅₀) namely the concentration value which could inhibit 50% of free radical activities (DPPH). DPPH is free radicals which are stable in a room temperature having electrons and hydrogen, and form a stable molecule. The absorbance of free radicals occurs when the antioxidant compounds which can give hydrogen atom have been bond to DPPH which results in the changes of violet colors into yellow colors. The absorbance of DPPH was measured by using spectrophotometer at the wavelength of 517 nm. Antioxidant activity on the pericarp, flesh, and seeds of the three species of rattan fruit and ascorbic acid as a positive control is displayed in Figure 1 to Figure 3.

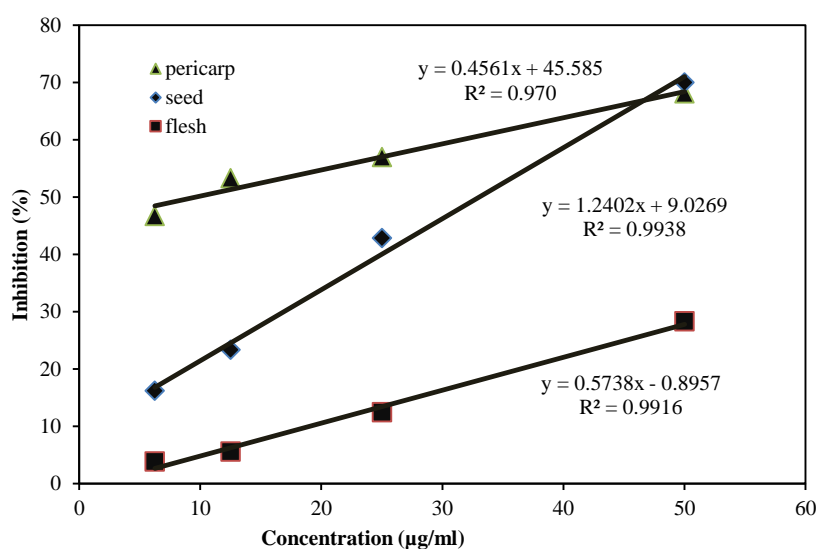


Figure 1. Antioxidant Activity in the Pericarp, Flesh and Seeds of *Calamus ornatus* Bl., and Ascorbic Acid

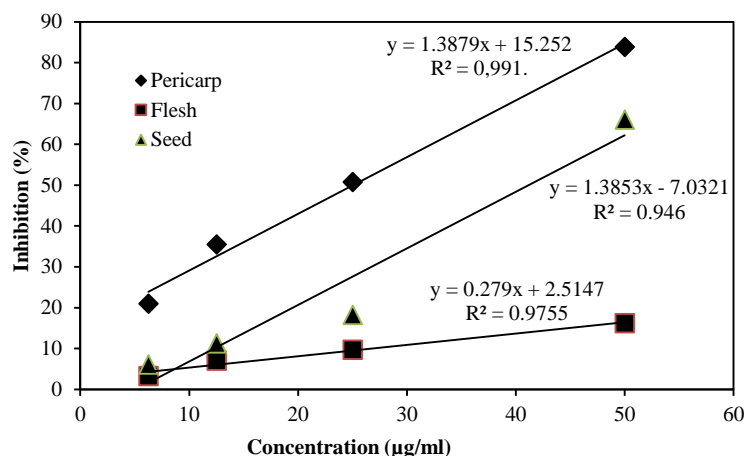


Figure 2. Antioxidant Activity in the Pericarp, Flesh and Seeds of *Calamus manan* Miq., and Ascorbic Acid

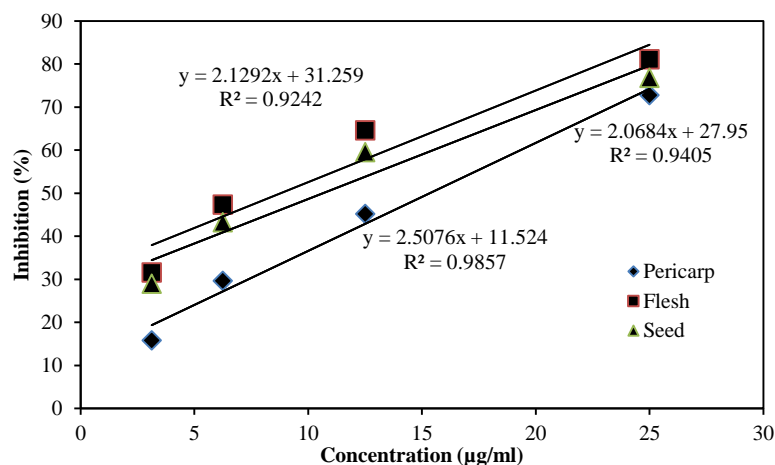


Figure 3. Antioxidant Activity in the Pericarp, Flesh and Seeds of *Calamus caesius* Bl., and Ascorbic Acid

Tabel 2. IC₅₀ values of fruit of three rattan species and ascorbic acid

Samples	IC ₅₀ (µg/ml)	Antioxidant Category*
<i>Calamus manan</i> Miq		
Pericarp	41.17	Strong
Flesh	170.19	Moderate
Seed	25.03	Strong
<i>Calamus caesius</i> Bl.		
Pericarp	15.34	Strong
Flesh	8.80	Strong
Seed	10.66	Strong
<i>Calamus ornatus</i> Bl.		
Pericarp	9.69	Strong
Flesh	88.82	Active
Seed	33.04	Strong
Ascorbic Acid	5.42	Strong

* IC₅₀ Value < 50 µg/ml Strong, 50 µg/ml - 100 µg/ml Active, 100 µg/ml - 250 µg/ml moderate, and > 500 µg/ml non active [14]

Furthermore, in order to identify the effectiveness of sample in inhibiting free radical, the value of IC_{50} was calculated which was obtained from linear regression equation (Figure 1, 2, and 3) which expressed the correlation between sample extract concentration as an x-axis and free radical inhibition as a y-axis. The results of calculation about the value of IC_{50} displayed on Table 2 showed that the three species of the fruit and the three different parts of the fruit had different antioxidant activities. The smaller the value of IC_{50} the stronger the antioxidant activities will be [14]. In the fruit of *C. manan* Miq., the power of antioxidant activities was successively ordered as follows: seeds > pericarp > flesh; in *C. caesius* Bl. flesh > seeds > pericarp, while in *C. ornatus* Bl. pericarp > seeds > flesh. If compared with ascorbic acid as a positive control toward the three type of fruit, it was found that ascorbic acid > *C. caesius* Bl. > *C. ornatus* Bl. > *C. manan* Miq. The antioxidant activities which were presented in order from the highest to the lowest activities can be illustrated as follows: the flesh of *C. caesius* Bl. (8.80 $\mu\text{g/ml}$) > the pericarp of *Calamus caesius* Bl. (9.69 $\mu\text{g/ml}$) > the seeds of *C. caesius* Bl (10.66 $\mu\text{g/ml}$) > the pericarp of *C. caesius* Bl (15.34 $\mu\text{g/ml}$) > the seeds of *C. manan* Miq (25.03 $\mu\text{g/ml}$) > the seeds of *Calamus ornatus* Bl. (33.04 $\mu\text{g/ml}$) > the pericarp of *Calamus manan* Miq (41.17 $\mu\text{g/ml}$) > the flesh of *Calamus ornatus* Bl. (88.82 $\mu\text{g/ml}$) > the flesh of *Calamus manan* Miq. (170.19 $\mu\text{g/ml}$) respectively. Ascorbic acid as a positive control has the highest antioxidant properties with IC_{50} values 5.42 $\mu\text{g/ml}$. Based on the results of the phytochemical assay, antioxidant activities mostly influenced by the existence of flavonoids, tannins and triterpenoids compounds, which were detected almost in all species of rattan fruit in this study. Flavonoids are

compounds which serve as antioxidant agents because their structure contains hydroxyl group which is able to give its hydrogen atom to free radical compounds so that it can inhibit a lot of oxidation reactions [15]. Jing [16] stated that the total flavonoids and phenol in *Rhododendron anthopogonoides* correlated significantly with antioxidant activities. A study on rattan fruit of *C. tenuis* Roxb was conducted by [5] showed that the total flavonoids correlated with the increasing antioxidant activities. Moreover, tannins as antioxidants have been discussed in the previous research findings. Rebaya [17] reported that there was a significant correlation between tannins, flavonoids and polyphenolic compounds and antioxidant activities in *Halimium halimifolium*. Bizuayehu [18] in their study also found that there was a positive correlation between tannins and antioxidant activities.

Triterpenoid is an antioxidant compound which serves as an antioxidant agent. Lin [19] stated that the increasing triterpenoid concentration will also increase the antioxidant activities in *Garcinia lucidum*. This indicates that triterpenoids play a role in increasing antioxidant activities. According to [20] triterpenoid content also strongly contributes to the antioxidant activities of mistletoe tea (*Scurrula atropurpurea* Bl. Dans).

CONCLUSIONS AND SUGGESTION

The fruit of *C. caesius* Bl has strong antioxidant activities in all parts of the tested fruit, while the fruits of *C. manan* Miq dan *C. ornatus* Bl. have strong antioxidant activities in its pericarp and seeds. Generally, the three species of rattan fruit have potentials as natural antioxidant agents based on the DPPH method, but the active compounds which play roles as

antioxidant agents have not identified yet so that it is necessary to conduct further studies concerning this issue.

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