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ANTIOXIDANT ACTIVITIES OF CRUDE METHANOLIC EXTRACT OF *Nephelium ramboutan-ake* (Labill.) Leenh. PEEL

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Abstract: *Nephelium ramboutan-ake* (Labill.) Leenh., Ngoh-khonsan in Thailand, is tropical species that is cultivated in Southeast Asia. The juicy, sweet pulps of its fruits are usually consumed fresh. In addition, its root has been used in traditional medicine for antihelmintic and antipyretic. In this research, the plant material was collected from East Kalimantan of Indonesia and antioxidant activities of crude methanolic extract of fruit peel were evaluated using five different methods including 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, hydroxyl radical scavenging assay, hydrogen peroxide (H_2O_2) scavenging assay, ferric reducing antioxidant power (FRAP) assay, and ferrous ion chelating assay. Ascorbic acid and EDTA were taken as standard. The total phenolic content (TPC) and total flavonoid content (TFC) of the plant extract were also determined using Folin-Ciocalteau and aluminum chloride method, respectively. The hydrogen peroxide scavenging activity of the plant extract was found to be slightly higher than that of ascorbic acid. The capability of scavenging DPPH radical and ferric ion reducing power of the plant extract were slightly less than those for ascorbic acid. On the other hand, the plant extract possessed less antioxidant activity against hydroxyl radical as compared with ascorbic acid and exhibited less ferric ion chelating capability than EDTA. TPC and TFC of the plant extract were found to be 306.0417 ± 0.6505 mg gallic acid equivalent/g crude extract and 14.0494 ± 0.1413 mg quercetin equivalent/g crude extract, respectively.

1. Introduction

Indonesia has 266 species of indigenous fruits, which most of them grow wild in the forests and few have been cultivated, and 144 of them were found in Kalimantan Island. The genus *Nephelium* is one of the four genera of native fruits cultivated in Indonesia. Kalimantan has 16 of 22 species of the genus *Nephelium* and one of them is *N. ramboutan-ake* (Labill.) Leenh. [1].

N. ramboutan-ake (Labill.) Leenh. has been widely known as ngoh-khonsan in Thailand. In Indonesia, it has been also known as rambutan ake, rambutan babat, rambutan sebabat, tenggaring, pulasan, kapulasan, tukou biawak, molaitomo, and mulitan [2]. It reaches 36 m in hight with short, wide and round stem. Its leaves are compound leaves with 1-7 strands per stalk. Small greenish flowers are in branched axillary or around the end of twigs. Its fruit is oval with purplish red rind and length 5-7.5 cm, watery yellowish white flesh flavored, and one seed inside [3-6]. Commonly *N. ramboutan-ake* (Labill.) Leenh. is not only grown wild in forest, but it is also cultivated for edible fruits [1,5]. Its roots and leaves are used as poultices, anthelmintic and to treat fever. Its roasted and boiled seeds can be consumed, as well as its dried seed kernels yield aromatic oil [4,6,7]. Many compounds were isolated and identified from the fruits of *N. ramboutan-ake* (Labill.) Leenh. Thirty-nine volatile compounds, mainly aliphatic hydrocarbons (70.2%), particularly pentadecane (61.4%), and aliphatic alcohols (19.4%) were identified by Wong *et al* [2]. Ikram *et al* [8] reported that the crude methanolic extract from the fruits of *N. ramboutan-ake* (Labill.) Leenh. contained phenolic compounds with high antioxidant activity. Chan *et al* [9] showed that the aqueous fraction of *N. ramboutan-ake* (Labill.) Leenh. peels could reduce the growth of human cancer cells.

However, the researches on the peel of *N. ramboutan-ake* (Labill.) Leenh. have been less found and there has been no reports on its total flavonoid content, phenolic content and antioxidant activities. Thus our present study has been focused on the content of phenolic and flavonoid compounds and the evaluation of antioxidant activities for the crude methanolic extract of *N. ramboutan-ake* (Labill.) Leenh. (abbreviated as CME).

2. Materials and Methods

2.1 Plant material and chemicals

N. ramboutan-ake (Labill.) Leenh. fruits were purchased from Pasar Malam Suryanata, Samarinda city, East Kalimantan Province, Indonesia on the 11th of January 2013. The fruit was identified by Dr. Medi Hendra, M.Si, Physiology Laboratory, Faculty of Mathematic and Natural Science of Mulawarman University. The voucher specimen (NR-DD-17) was deposited in the Forest Products Technology Laboratory, Forestry Faculty of Mulawarman University. 2,2-diphenyl-1-picrylhydra-zyl (DPPH), ascorbic acid, aluminium chloride hydrate, trichloro-acetic acid, and gallic acid were purchased from Sigma Aldrich GmbH (Germany). Folin-ciocalteu reagent, hydrogen sodium 4,4'-[3-(2-pyridinyl)-1,2,4-triazine-5,6-diyl]dibenzene-sulfonate (ferrozine), iron(II) sulfate heptahydrate, iron(III) chloride, potassium dihydrogen phosphate, potassium sodium hydrogen phosphate, quercetine,

were purchased from Merck KGaA (Germany). Eethylenediaminetetraacetic acid (EDTA), 35% hydrogen peroxide, potassium hexacyano-ferrate(III), salysilic acid, sodium bicarbonate, were purchased from Ajax Finechem (Australia).

2.2 Preparation of crude methanolic extract (CME)

N. ramboutan-ake (Labill.) Leenh. fruits were washed with running tap water to remove the dirt and then were left until they dried at least 30 minutes. Its peels were separated out from its seeds and flesh. They were cut into small pieces and 1.23 Kg of the small pieces of the peels was extracted with 3.5 liters methanol three times for 48 hours. The methanolic extract was concentrated with a rotary evaporator at 39^{o}C to obtain crude methanolic extract (CME) with the percent yield of 6.4%.

2.3 Determination of total phenolic content and flavonoid content

2.3.1 Total phenolic content

Total phenolic content (TPC) was estimated by Follin Ciocalteau method described by Wang *et al* with a little modification [10]. A volume of 0.2 ml of 0.1 mg/ml tested sample was mixed with 1.0 ml of 0.2 N Folin-Ciocelteau reagent. The mixture was allowed to stand under room temperature for 5 minutes and then 0.8 ml of 7.5% Na_2CO_3 was added to the mixture. After incubation for 2 hours at room temperature, the absorbance of the reaction mixture was measured at 760 nm using UV-Vis spectrophotometer. Total phenolic content was calculated from the standard graph of gallic acid and the results were expressed as milligram gallic acid equivalent (mg/g crude extract).

2.3.2 Total flavonoid content

Total flavonoid content (TFC) was determined by following the procedure of Wang *et al* [10]. A volume of 1 ml of 0.5 mg/ml of tested sample was mixed with 1 ml of 2% $AlCl_3$ methanol solution. The mixture was allowed to stand for 1 hour under room temperature, and then the absorbance of the reaction mixture was measured at 420 nm using UV-Vis spectrophotometer. The content of flavonoids was calculated using standard graph of quercetin and the results were expressed as milligram quercetin equivalent (mg/g crude extract).

2.4 *In vitro* antioxidant assays

2.4.1 DPPH radical scavenging activity

DPPH radical scavenging assay was performed according to the method of Wang *et al* with a few modifications [10]. A volume of 1 ml of each concentration of tested sample dissolved in methanol was mixed with 1.0 ml of 0.1 mM DPPH methanolic solution. The mixture was left in the dark for 20 minutes and the absorbance of the reaction mixture was measured at 517 nm using UV-Vis spectrophotometer against a blank. Ascorbic acid (AA) was used as the control. The percentage of DPPH scavenging activity was calculated as

Scavenging activity = $[1-((A_1-A_2)/A_0)]x100\%$

where A_0 was the absorbance of control (without the tested sample), A_1 was the absorbance in the presence of the tested sample, and A_2 was the absorbance without DPPH.

2.4.2 Hydrogen peroxide scavenging activity

The H_2O_2 scavenging activity assay was evaluated by following to the method of Jiang *et al* with a few modifications [11]. A volume of 0.5 ml each concentration of tested sample dissolved in methanol was mixed with 1.2 ml of 0.1 M phosphate buffer (pH = 7.4) and 0.3 ml of 40 mM H_2O_2 in phosphate buffer. The mixture was allowed to stand for 10 minutes under room temperature. The absorbance of the reaction mixture was measured at 230 nm using UV-Vis spectrophotometer against a blank. Ascorbic acid (AA) was used as the control. The percentage of H_2O_2 scavenging activity was calculated as

Scavenging activity = $[1-((A_1-A_2)/A_0)]x100\%$

where A_0 was the absorbance of control (without the tested sample), A_1 was the absorbance in the presence of the tested sample, and A_2 was the absorbance without H_2O_2.

2.4.3 Ferric reducing antioxidant power (FRAP) activity

The FRAP assay was performed using the method of Kilic *et al* with a few modifications [12]. A volume of 0.3 ml of each concentration of tested sample dissolved in methanol was diluted in 0.5 ml of 0.2 M phosphate buffer (pH = 6.6). 0.5 ml of 1% potassium hexacyanoferrate(III) was then added to the mixture and followed by vigorous shaking. The mixture was incubated in thermostat at 50^{o}C for 20 minutes. After incubation, it was added with 0.5 ml of 10% trichloroacetic acid and 0.2 ml of 0.1% ferric chloride. The absorbance of the reaction mixture was measured at 700 nm using UV-Vis spectrophotometer. Increased absorbance of the reaction mixture indicated increased reducing power activity of tested sample. Ascorbic acid (AA) was used as the control.

2.4.4 Ferrous chelating activity

The Fe(II)-chelating activity assay was determined according to the method of Xu *et al* with minor modifications [13]. A volume of 0.75 ml of each concentration of tested sample dissolved in 1:1 of methanol and deionized water was mixed with 0.75 ml of 0.1 mM ferric sulfate and followed with shaking. After incubation for 1 minute, 0.75 ml of 0.25 mM ferrozine was added and kept for 10 minutes at room temperature to complex the residual Fe^{2+}. The absorbance of the Fe^{2+}-ferrozine complex was measured at 562 nm using UV-Vis spectrophotometer. EDTA was used as the control. The percentage of Fe^{2+} scavenging activity was calculated as

Scavenging activity = $[1-((A_1-A_2)/A_0)]x100\%$

where A_0 was the absorbance of control (without the tested sample), A_1 was the absorbance in the presence of the tested sample, and A_2 was the absorbance without ferrozine.

2.4.5 Hydroxyl radical scavenging activity

The hydroxyl radical scavenging assay was determined by the method of Du *et al* with a few modifications [14]. The reaction mixture contained 0.5 ml of each concentration of tested sample dissolved in methanol, 0.5 ml of 4 mM ferric sulfate in deionized water, 0.5 ml of 4 mM salicylic acid in methanol, and 0.5 ml of 4 mM H_2O_2. The mixture was shaken and incubated in thermostat at 37°C for 30 minutes. After incubation, the absorbance of the hydroxylated salicylate complex was measured at 510 nm using UV-Vis spectrophotometer. Ascorbic acid (AA) was used as the control. The percentage of hydroxyl radical scavenging activity was calculated as

Scavenging activity = $[1-((A_1-A_2)/A_0)]x100\%$

where A_0 was the absorbance of control (without the tested sample), A_1 was the absorbance in the presence of the tested sample, and A_2 was the absorbance without H_2O_2.

2.5 Statistical analysis

All the experiments were performed in triplicate. The experimental results are expressed as mean ± standard deviation of mean (SDM). Statistical analyses were performed using Student's *t*-test and one-way analysis of variance (ANOVA) for the triplicate ($n=3$) test data.

3. Results and Discussion

3.1 Extract yield, total phenolic and flavonoid contents

CME was obtained as high viscous and dark brown liquid, and the yield of CME was 6.4% (w/w). TPC and TFC of the plant extract were found to be 306.0417 ± 0.6505 mg gallic acid equivalent/g of the crude extract and 14.0494 ± 0.1413 mg quercetin equivalent/g of the crude extract, respectively.

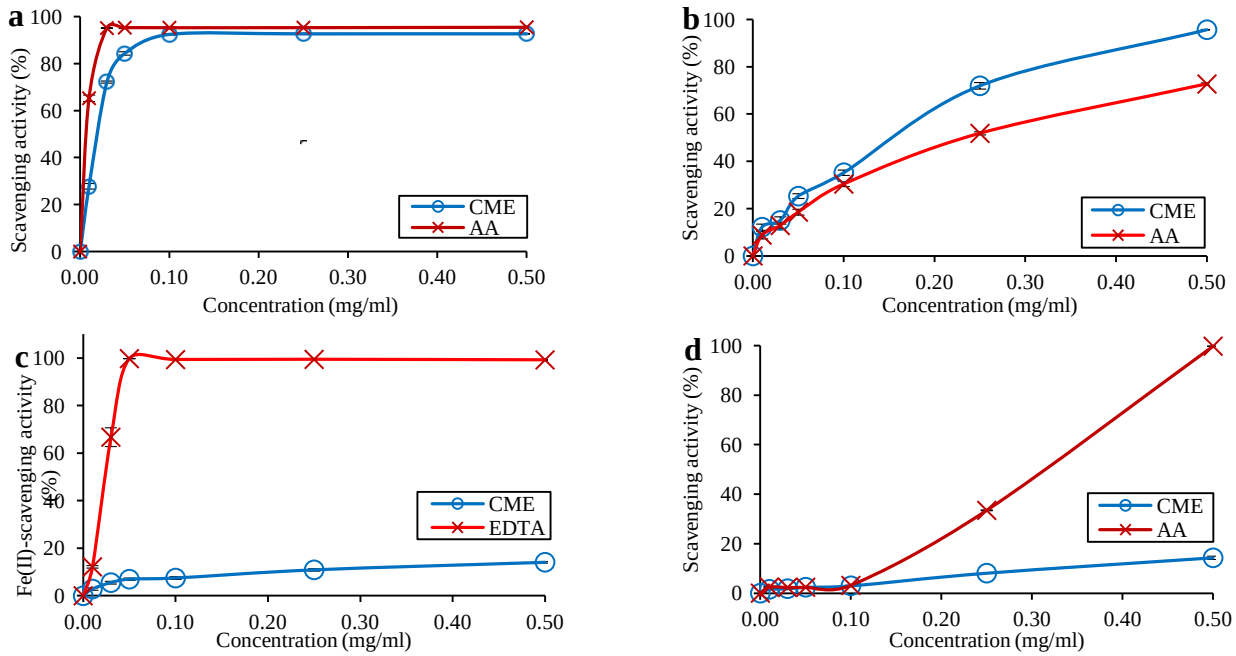

Figure 1. (a) The DPPH-radical scavenging activity; (b) H_2O_2 scavenging activity; (c) Fe(II)-chelating activity; (d) OH-radical scavenging activity of CME. The absorbance values of each tested sample were converted to % scavenging activity and data were plotted as the means of replicate % scavenging activity values ± SDM ($n=3$) against concentration of tested sample in mg/ml unit.

Table 1: *In vitro* antioxidant activity of CME and controls

Sample/ Control	IC_{50} (mg/ml)		Antioxidant activities at 0.5 mg/ml concentration of tested		
	DPPH radical scavenging activity	Hydrogen peroxide scavenging activity	Ferrous ion chelating activity (%)	Hydroxyl radical scavenging activity (%)	FRAP activity (Abs)
CME	0.0190 ± 0.0004	0.1530 ± 0.046	14.4793 ± 0.3354	14.2750 ± 0.6726	0.8128 ± 0.0029
AA	0.0066 ± 0.0003*	0.2333 ± 0.068	-	99.7325 ± 0.0003*	0.8251 ± 0.0076
EDTA	-	-	99.7258 ± 0.0489*	-	-

The value are expressed as mean ± SDM ($n=3$). *$p<0.01$, compared with CME

3.2 *In vitro* antioxidant activity

3.2.1 DPPH radical scavenging activity

DPPH radical scavenging activity assay is used to evaluate the capability of the sample as hydrogen donor to DPPH radicals [15]. As seen in Figure 1a, ascorbic acid showed an excellent scavenging activity with an IC_{50} value of 0.0066 ± 0.0003 mg/ml. It was obvious that CME had significantly less DPPH radical scavenging activity with an IC_{50} value of 0.0190 ± 0.0004 mg/ml than ascorbic acid standard about twice.

3.2.2 Hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging activity assay is used to evaluate the capability of the sample to inhibit the H_2O_2 decomposition reaction to produce reactive hydroxyl radicals. In the cell, H_2O_2 can react with Fe^{2+} or the superoxide anion radical to generate hydroxyl radical [10,16]. Compared to ascorbic acid, CME was more effective to scavenge H_2O_2 than ascorbic acid insignificantly. Figure 1b showed both of the CME and ascorbic acid significantly scavenged the H_2O_2 with increase in concentrations of tested samples.

3.2.3 Ferrous ion chelating activity

Iron and copper are essential metals in the human body for some activities including cellular respirations, oxygen transportations, and redox reactions [15]. In the form of cations, Fe^{2+} and Cu^{2+}, ions participate in Fenton reaction to produce reactive hydroxyl radicals from H_2O_2 and in the alkyl peroxide decomposition to produce reactive alkoxyl and hydroxyl radicals [15,16]. In this study, ferrozine can form complexes with ferrous ions. In the present of chelating agents, the formation of the Fe^{2+}-ferrozine complex with red color is interrupted and the red color of the complex is consequently decreased. Thus the extent the color reduction is an indicator that can be used to estimate the Fe^{2+}-chelating ability of tested sample. Figure 1c showed the CME has much less chelating effect as compared to EDTA. At the maximum concentration tested (0.5 mg/ml), the percentage of Fe^{2+}-chelating activity for CME and EDTA was 14.4793 ± 0.3354 % and 99.7258 ± 0.0489 %.

3.2.4 Hydroxyl radical scavenging activity

Hydroxyl radical is the most reactive oxygen species among all ROS. It can react with various bimolecules in living cells by hydrogen abstraction, double bond addition, electron transfer and radical formation, etc [17,18]. Hydroxyl radical scavenging activity of CME was determined as the percentage of inhibition of hydroxyl radicals generated from the Fenton reaction mixture. Figure 1d showed the CME exhibited much lower scavenging activity as compared to ascorbic acid. At the maximum concentration tested (0.5 mg/ml), the percentage of hydroxyl radical scavenging activity for CME and ascorbic acid was 14.2750 ± 0.6726 % and 99.7325 ± 0.0003 %.

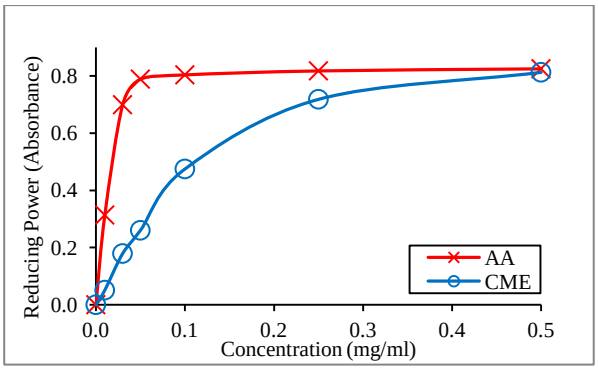

Figure 2. Reducing power of CME. Data were plotted as the means of replicate of absorbance values ± SDM ($n=3$) against CME concentration in mg/ml unit.

3.2.5 FRAP activity

The capacity of a reducing agent to donate its electron to free radical is a way to neutralize the radical by forming the stable product [10,12]. As shown in Figure 2, the CME was found to possess high reducing power, although ascorbic acid was better. The reducing power of CME and ascorbic acid were 0.8128 ± 0.0029 and 0.8251 ± 0.0076 at the concentration 0.5 mg/ml, statistically, there is no differential between CME and ascorbic acid.

4. Conclusions

In this study, we have discussed the *in* vitro antioxidant activities of the crude methanolic extract of *N. ramboutan-ake* (Labill.) Leenh. peels (CME) against DPPH and hydroxyl radicals, hydrogen peroxide, and capability of chelating Fe^{2+} ion and reducing Fe^{3+} ion. The experimental results obtained suggest that CME possessed various antioxidant activities, which are related to the controls (ascorbic acid and EDTA). Thus, *N. ramboutan-ake* (Labill.) Leenh. can be considered as a promising source of potential antioxidants.

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