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Research Article

Mangosteen (*Garcinia mangostana* L.): Evaluation of *In Vitro* Antioxidant Activities

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ABSTRACT

Introduction: Mangosteen (*Garcinia mangostana* L.), is an evergreen of the *Guttiferae* family that carries antioxidant activity. **Objectives:** to examine the antioxidant activity of the leaves, branches and rinds of the mangosteen using DPPH and CUPRAC methods, total phenolic content (TPC) and total flavonoid content (TFC), analyze the correlation between TPC, TFC and antioxidant activity, the correlation between two methods, and found the levels of flavonoid compounds. **Methods:** Extraction was performed by reflux method using solvents with graded polarity, namely n-hexane, ethyl acetate and ethanol. Determination of antioxidant activity with DPPH and CUPRAC, TPC and TFC were performed by UV-visible spectrophotometer. The correlation between TPC, TFC and antioxidant activity of DPPH and CUPRAC as well as the correlation between two methods were conducted by Pearson's method. The level of flavonoid compounds was performed by HPLC**. Results:** Mangosteen leaves, branches and rinds extracts had antioxidant activity of DPPH in the range of 39.920 – 489.708 mg AAE/g and antioxidant activity of CUPRAC in the range of 116.360 – 570.400 mg AAE/g. The highest TPC was given by the ethanol leaves extract (49.525 ± 4.263 g GAE/100 g) and the highest TFC was given by the n-hexane rinds extract (13.859 ± 1.451 g QE/100 g). The ethanol rinds extract contained rutin 0.0327% and kaempferol 0.0049%. **Conclusions:** TPC and TFC correlated positive and significant with the value of antioxidant activity, except for the n-hexane leaves extract using the DPPH method. The DPPH and CUPRAC methods gave linear results in determining the antioxidant activity of mangosteen extracts.

Key words: Antioxidant, Mangosteen, Leaves, Branches, Rinds, DPPH, CUPRAC.

INTRODUCTION

Free radicals are unstable molecules with one or more unpaired electrons in their outermost orbital. In order for them to be stable, these molecules will find their electron pairs by taking electrons from other molecules, which explains why free radicals are also known as reactive oxygen species (ROS). Excess number of free radicals can trigger oxidative stress which will cause oxidative damage at the cellular, tissue and organ levels. Free radicals can come from within the body (endogenous sources) or from outside the body (exogenous sources). Endogenous sources include phagocytic cells, mitochondria, endoplasmic reticulum and peroxisomes. Meanwhile, exogenous sources include alcohol, cigarette smoke, pollution, pesticides, heavy metals, transition metals, industrial solvents, radiation and the consumption of certain drugs such as halothane and paracetamol.[1]

Antioxidants are compounds that can counteract free radicals in the body by complementing unpaired electrons, so that chain reactions due to the presence of free radicals can be inhibited. The human body can produce endogenous antioxidants such as the enzyme superoxide dismutase, catalase and glutathione, but the intake of natural antioxidants is still needed. Sources of natural antioxidants come from vegetables, fruit, vitamins A, C, E, polyphenolic compounds such as flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, catechins, isocatechins, epicatechins and phenolic acids.[2]

Mangosteen (*Garcinia mangostana* L.), also known as the 'queen of fruit', is an evergreen of the *Guttiferae* family that is distributed throughout Indonesia, Malaysia, India, Myanmar, Thailand, the Philippines and Sri Lanka. In these countries, the rind and flesh of the mangosteen fruit are used as traditional medicines to treat abdominal pain, diarrhea, dysentery, infection due to wounds, pus and chronic ulcers.[3] The mangosteen fruit component consists of 70-75% rinds, 10-15% flesh, and 15-20% seeds. Mangosteen rinds has high antioxidant activity due to its abundant contents of polyphenolic compounds such as anthocyanins, tannins, xanthones and phenolic acids.[4] Besides having an antioxidant activity, this plant has various pharmacological activities such as antimicrobial, antimalarial, anti-inflammatory, antidiabetic,[5] antiviral, analgesic, antihistamine, antiobesity, antidepressant, antifungal, antimutagenic, antitumor, anticancer and antiproliferative.[6]

The objectives of this research were to examine the antioxidant activity of the leaves, branches and rinds of the mangosteen (*Garcinia mangostana* L.) using the DPPH and CUPRAC methods, to determine total phenolic content (TPC) and total flavonoid content (TFC), to analyze the correlation between TPC and TFC to the antioxidant activity values from both of the method, the correlation between two methods in sample extracts, and found the levels of flavonoid compounds in extract.

MATERIALS AND METHODS

Materials

Gallic acid, quercetin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), neocuproine, ascorbic acid, cupric chloride,

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n-hexane, ethyl acetate, ethanol, methanol, sodium acetate, ammonium acetate, sodium carbonate, aluminum (III) chloride, Folin-Ciocalteu reagent and aquadest.

Preparation of sample

The process of materials collection and preparation includes the collection of leaves (L), branches (B) and rinds (R) of the mangosteen fruit (*Garcinia mangostana* L.), sorting the materials, washing the materials, cutting the materials, drying the ingredients using an oven to make crude drug and grinding the crude drug to form a powder. Then the powder was stored in a dry and closed container. The leaves, branches and rinds of the mangosteen fruit were obtained from Kaluang Tapi Street, Nagari Koto Tangah, Tilatang Kamang District, Agam Regency, Bukittinggi City, West Sumatra Province-Indonesia.

Extraction

Extraction of three hundred grams crude drug powder was performed by hot method, the reflux method, using solvents with graded polarity, namely n-hexane as the nonpolar solvent, ethyl acetate as the semipolar solvent and ethanol as the polar solvent. The extraction was repeated three times for each solvent and the process in one time extraction was performed in 2 - 3 hours after the solvent boiled. The extract obtained was then concentrated using a rotary evaporator and placed on a water bath until a thick extract was obtained. Thus, there were nine extracts: three n-hexane extracts (named as L1, B1 and R1), three ethyl acetate extracts (L2, B2 and R2), and three ethanol extracts (L3, B3 and R3).

Total phenolic content (TPC) determination

Determination of total phenolic content was adopted from Pourmorad[7] using Folin-Ciocalteu reagent. The standard solution used was gallic acid in the concentration range of 60 - 130 µg/mL. The blank solution contained 50 µL methanol, 500 µL of Folin-Ciocalteu reagent 10%, and 400 µL of 1 M Na_2CO_3 which were mixed in an Eppendorf tube. The standard solution was prepared by adding gallic acid 50 µL and a blank solution. Sample solution was prepared by adding 50 µL of each extract and blank solution into an Eppendorf tube. After all mixtures were incubated for 15 min at room temperature, the absorbance was measured by UV-visible spectrophotometer at λ 765 nm. Absorbance measurements were repeated six times for each extract. The gallic acid calibration curve was obtained from the absorbance of each concentration of the standard solution. The total phenolic content was expressed in g gallic acid equivalent per 100 g extract (g GAE/100 g).

Total flavonoid content (TFC) determination

Determination of the total flavonoid content was adopted from Chang[8] using quercetin in the concentration range of 40 - 110 µg/mL that were dissolved in the blank as a standard solution. The blank solution contained 300 µL of methanol, 20 µL of 10% $AlCl_3$, 20 µL of 1 M sodium acetate and 560 µL distilled water which were mixed in an Eppendorf tube. Sample solution was prepared by adding 100 µL of each extract and blank solution. After all mixtures were incubated for 30 min at room temperature, the absorbance was measured by UV-visible spectrophotometer at λ 415 nm. Absorbance measurements were repeated six times for each extract. The quercetin calibration curve was obtained from the absorbance of each concentration of the standard solution. The total flavonoid content was expressed in g of quercetin equivalent per 100 g of extract (g QE/100 g).

Antioxidant activity determination using DPPH method

Antioxidant activity determination using the DPPH method used ascorbic acid as a standard, DPPH stock solution as a control, and pro-analytical methanol as a blank. DPPH stock solution was made with a concentration of 50 µg/mL. Standard stock solution was prepared by dissolving 20 mg of ascorbic acid in 100 mL of pro-analytical methanol, then 10 µL, 12.5 µL, 15 µL, 20 µL, 25 µL and 30 µL were taken and diluted using pro-analytical methanol until 125 µL. Furthermore, 750 µL of DPPH solution was added and then incubated for 30 min. The absorbance was measured by UV-visible spectrophotometer at λ 517 nm. From the absorbance measurement, the percentage of DPPH scavenging activity from each ascorbic acid concentration will be obtained and be turned into a calibration curve. The regression equation obtained must at least have a value of $R^2 = 0.99$.

Sample solution was prepared by dissolving the extract in pro-analytical methanol. A total of 12.5 µL of sample solution was added by pro-analytical methanol until 125 µL of and DPPH solution 750 µL in Eppendorf tube then incubated for 30 min. The absorbance was measured at λ 517 nm by UV-visible spectrophotometer. Absorbance measurements were repeated six times for each extract. The percentage of DPPH scavenging activity by sample that obtained is then entered into the regression equation of the ascorbic acid calibration curve. The antioxidant activity was expressed as the equivalency of ascorbic acid, namely mg ascorbic acid equivalent (AAE)/g sample.[9]

Antioxidant activity determination using CUPRAC method

Antioxidant activity determination using the CUPRAC method used ascorbic acid as a standard, CUPRAC stock solution as a control and ammonium acetate buffer as a blank. CUPRAC stock solution was made with a concentration of 100 µg/mL as much as 100 mL consisting of $CuCl_2.H_2O$ solution, neocuproin solution and ammonium acetate buffer. As much as 20 mg of ascorbic acid was dissolved in 100 mL of pro-analytical methanol, then 15 µL, 17.5 µL, 20 µL, 22.5 µL, 25 µL and 27.5 µL were taken and diluted using ammonium acetate buffer until 250 µL. Then, 750 µL of CUPRAC solution was added and then incubated for 30 min. The absorbance was measured at λ 450 nm using a UV-visible spectrophotometer. The CUPRAC capacity was measured as the percent increase in the absorbance from each concentration of ascorbic acid and will be converted into a calibration curve. The regression equation obtained must at least have a value of $R^2 = 0.99$.

Sample solution was prepared by dissolving the extract in pro-analytical methanol. A total of 12.5 µL of sample solution was added by ammonium acetate buffer until 250 µL and CUPRAC solution 750 µL in Eppendorf tube and then incubated for 30 min afterwards. The absorbance was measured at λ 450 nm by UV-visible spectrophotometer. Absorbance measurements were repeated six times for each extract. The percentage of CUPRAC capacity obtained was then entered into the regression equation of the ascorbic acid calibration curve. The antioxidant activity was expressed as with ascorbic acid, namely mg ascorbic acid equivalent (AAE)/g sample.[10]

Statistical analysis

Analysis of each sample was repeated six times and statistical analysis was carried out by the utilization of Minitab 19 software. The results were analyzed using one-way ANOVA - Tukey (p value < 0.05). All of the results expressed in means ± standard deviation. The correlation analysis between the TPC and TFC on the value of the antioxidant activity of DPPH and CUPRAC as well as the correlation of the two methods were conducted by Pearson's method.

The levels of flavonoid compounds determination

Determination of flavonoid compounds levels was carried out using the HPLC method on the extract with the largest yield (ethanol rinds extract), using rutin and kaempferol as standard compunds. The HPLC used was HPLC-20AD with Shimadzu SPD-20A UV/Vis detector at λ 360 nm. The stationary phase used was LiChrospher® 100 RP-C18 (Length 100 mm, diameter 4 mm, 20 mm precolumn (Merck)) with water (eluent A) and methanol (eluent B) as mobile phases. A linear gradient system of 40% to 60% eluent B was used for 5 min, then a

gradient of 70% eluent B until the 10th min, and a gradient of 40% eluent B until the 15th min. The analysis was carried out at a flow rate of 1 mL/min (CTO-20A pump, Shimadzu, Japan), the injection volume was 20 µL, and the column temperature was set at 30 °C (Oven CTO-20A, Shimadzu, Japan). The standard compounds used was rutin 20 µg/mL and kaempferol 5 µg/mL, while the ethanol rinds extract of mangosteen was prepared at 100,000 µg/mL. The measurement of compound level was calculated using the one-point method by comparing the area under the curve (AUC) with the concentration of the sample extract and each standard.

RESULTS

Total phenolic and flavonoid content

Total phenolic content (TPC) in various extracts were expressed in gallic acid equivalent (GAE) using the calibration curve equation y = 0.0057x - 0.0774; R^2 = 0.9916. TPC in various extracts of mangosteen leaves, branch and rinds were presented in Figure 1. The highest TPC in this experiment was showed by ethanol leaves extract (L3) (49.525 g GAE/100 g). The total flavonoid content (TFC) among various extracts were determined using quercetin calibration curve equation y = 0.0069x + 0.0167; R^2 = 0,9986 as quercetin equivalent (QE). TFC in various extracts of mangosteen leaves, branch and rinds were exposed in Figure 1. The highest TFC in this experiment was exposed by n-hexane rinds extract (P1) 13.859 g QE/100 g.

Antioxidant activity using DPPH method

The determination of antioxidant activity with DPPH assay was using 2,2-diphenyl-1-picrylhydrazyl (DPPH) as free radicals. The antioxidant activity values of mangosteen extract from leaves, branches and rinds were shown at Figure 2 and stated as mg ascorbic acid equivalent (AAE)/g sample.

Antioxidant activity using CUPRAC method

The determination of antioxidant activity with CUPRAC assay was using neocuproin as the ligand. The antioxidant activity values of mangosteen extract from leaves, branches and rinds were demonstrated at Figure 2 and stated as mg ascorbic acid equivalent (AAE)/g sample.

Correlation between TPC and TFC to the antioxidant activity

The correlation analysis between TPC and TFC in the extract of leaves, branches and rinds of mangosteen on antioxidant activity was carried out by Pearson's method. TPC and TPC were declared to have a contribution if they gave a positive and significant correlation to the antioxidant activity value. The results were shown in Table 1.

Correlation between DPPH and CUPRAC methods

The correlation between antioxidant activity with two methods in the extract of leaves, branches and rinds of mangosteen were also

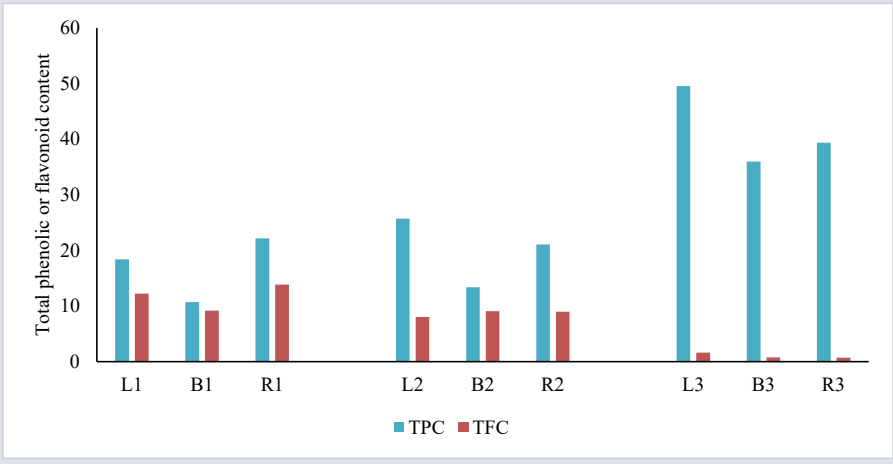

Figure 1: Total phenolic (g GAE/100 g) and flavonoid content (g QE/100 g) of mangosteen extracts.

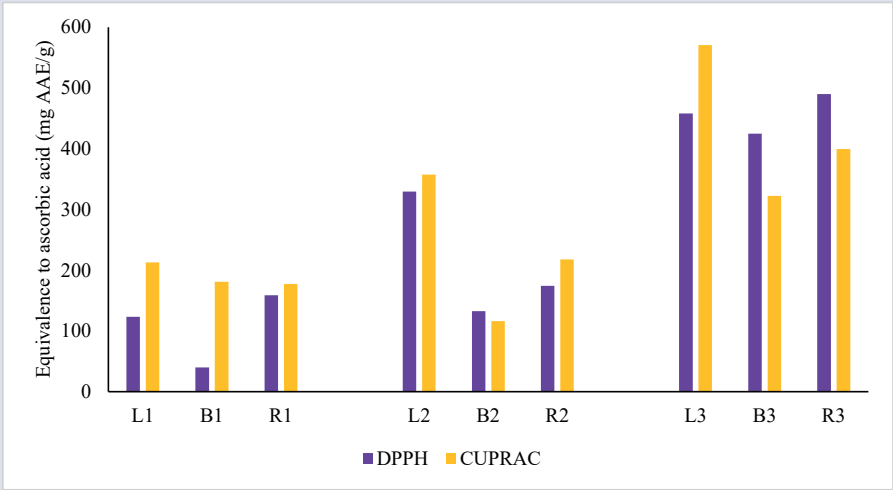

Figure 2: Antioxidant activities of DPPH and CUPRAC methods of mangosteen extracts.

determined statistically using Pearson's method. The results were represented in Table 2.

The levels of flavonoid compounds determination

AUC of the standards (rutin and kaempferol) for determination the levels of flavonoid compounds on ethanol rinds extract were exposed on the chromatogram in Figure 3 and Table 3.

DISCUSSION

The previous researches[11-12] reported that *Garcinia mangostana* had antioxidant activity. There were no study regarding antioxidant activity of different extracts (n-hexane, ethyl acetate and ethanol) of leaves, branch and rinds from *G. mangostana* L. which was grown in Bukittinggi, West Sumatra-Indonesia, using DPPH and CUPRAC methods.

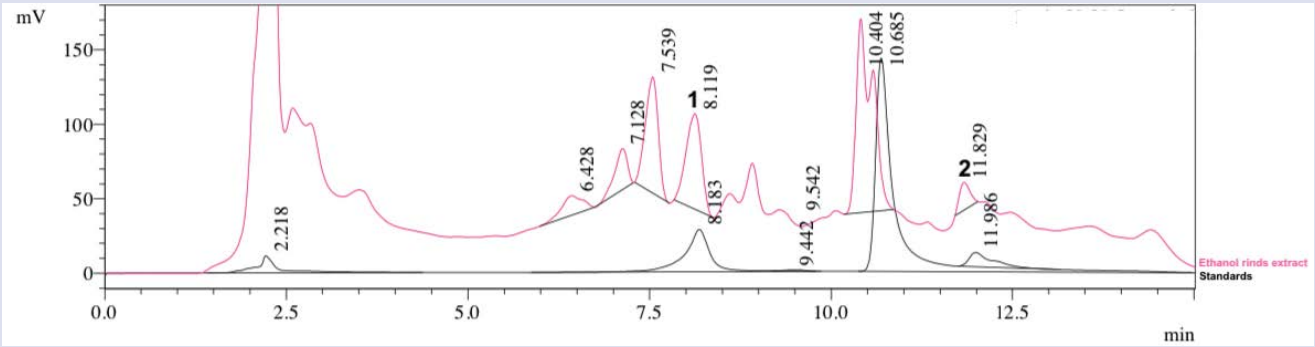

Figure 3: HPLC chromatogram of ethanol rinds extract and standards, 1: rutin, 2: kaempferol, black line chromatogram = standard, pink line chromatogram = sample.

Table 1: Correlation of the TPC and TFC of *G. mangostana* extracts with antioxidant activity.

Antioxidant parameter	Pearson's coefficient correlation (r)	
	TPC	TFC
DPPH L1	0.415^{ns}	0.319^{ns}
DPPH B1	0.796**	0.972**
DPPH R1	0.975**	0.948**
DPPH L2	0.939**	0.801**
DPPH B2	0.992**	0.784**
DPPH R2	0.941**	0.965**
DPPH L3	0.881**	0.994**
DPPH B3	0.934**	0.990**
DPPH R3	0.993**	0.921**
CUPRAC L1	0.934**	0.839**
CUPRAC B1	0.856**	0.973**
CUPRAC R1	0.979**	0.934**
CUPRAC L2	0.959**	0.881**
CUPRAC B2	0.933**	0.940**
CUPRAC R2	0.874**	0.964**
CUPRAC L3	0.783**	0.934**
CUPRAC B3	0.967**	0.989**
CUPRAC R3	0.839**	0.877**

ns = not significant, ** = significant at $p < 0.01$

ns = not significant, ** = significant at $p < 0.01$

Table 2: Correlation between DPPH and CUPRAC methods.

Antioxidant parameter	Pearson's coefficient correlation (r)								
	CUPRAC L1	CUPRAC B1	CUPRAC R1	CUPRAC L2	CUPRAC B2	CUPRAC R2	CUPRAC L3	CUPRAC B3	CUPRAC R3
DPPH L1	0.912**								
DPPH B1		0.969**							
DPPH R1			0.959**						
DPPH L2				0.966**					
DPPH B2					0.921**				
DPPH R2						0.971**			
DPPH L3							0.932**		
DPPH B3								0.969**	
DPPH R3									0.844**

** = significant at $p < 0.01$

** = significant at $p < 0.01$

Table 3: Retention time and AUC data for determination of flavonoid compound levels.

Flavonoid	Retention time (min)		AUC		Compound Levels (%)
	Standard	Sample	Standard	Sample	
Rutin	8.183	8.119	581505	951039	0.0327
Kaempferol	11.986	11.829	191531	187714	0.0049

The antioxidant activity assay is divided into three categories based on the mechanism of action, namely hydrogen transfer, electron transfer and combined mechanism. The combined mechanism includes hydrogen transfer, electron transfer and electron-proton transfer in various proportions based on the reaction conditions. DPPH is an example of a method that can be used to determine antioxidant activity based on a combined mechanism, while CUPRAC is based on electron transfer.[13]

DPPH is a free radical in the form of a monomer that is soluble in organic solvents such as methanol and ethanol, but insoluble in water. The DPPH method will determine the percentage of DPPH scavenging activity by sample using the various concentration. This method is based on electron transfer from antioxidants to neutralize DPPH radicals which are characterized by color shifting from dark purple to pale yellow. DPPH will be reduced so that there is a change in color intensity which is proportional to the number of H atoms followed by a decrease in the absorbance value of DPPH. The smaller the absorbance, the greater the percentage of DPPH scavenging activity decreased by the sample.[13]

Based on the present study of antioxidant activity assay using the DPPH method on the n-hexane extract, it was shown that the highest antioxidant activity was given by n-hexane rinds extract (R1) (158.566 ± 2.504 mg AAE/g). In the ethyl acetate extract, samples that had the highest antioxidant activity were found in the leaves (L2) (329.232 ± 23.940 mg AAE/g). Meanwhile, the ethanol extract was found to have the highest antioxidant activity in the rinds (R3) (489.708 ± 13.829 mg AAE/g). It can be seen in Figure 2, the test results for the highest antioxidant activity of each sample were found in the ethanol extract and antioxidant activity from the DPPH method of various extracts had a different result in the range of 39.920 to 489.708 mg AAE/g.

The results of a previous study by Palakawong[11] on the antioxidant activity of the leaves, stems and rinds of mangosteen that were extracted using 50% ethanol, showed the DPPH antioxidant activity values in IC_{50} were 9.44 ± 0.39 µg/mL for leaves, 6.46 ± 0.36 µg/mL for stems and 5.94 ± 0.14 µg/ mL for rinds. In addition, from the previous research by Tjahjani[14] on the antioxidant activity of mangosteen rinds using the DPPH method, revealed that the DPPH antioxidant activity value in IC_{50} was 7.48 ± 0.19 µg/mL for samples that were extracted by maceration with 96% ethanol and 6.56 ± 0.31 µg/mL for samples that were extracted by maceration with 70% ethanol. IC_{50} is the concentration of the test sample that causes a 50% decrease in DPPH activity. That is, the greater the IC_{50} value, the smaller the percentage of DPPH scavenging activity by the sample. Based on previous researchers, it is proven that the mangosteen rinds had better antioxidant activity than the other parts of plant. Research on antioxidant activity using the DPPH method by Chaovanalikit[15] was carried out on the flesh, outer pericarp and inner pericarp of the mangosteen by extraction using acetone and the residue obtained was dissolved in 0.1% HCl. The obtained EC_{50} values were 133.33 ± 25.17 µg/mL for flesh, 4.73 ± 0.55 µg/mL for outer pericarp, and 1.35 ± 0.13 µg/mL for inner pericarp. Jaisupa[16] also conducted research on mangosteen rinds using the DPPH method. The rinds of mangosteen was macerated using ethanol with low temperature heating and then concentrated using a rotary evaporator. The IC_{50} value was 26.75 ± 3.07 µg/mL. Research on antioxiant activity using the DPPH method by Ghazemzadeh[17] on mangosteen rinds was carried out using a microwave extraction method. The experiment was carried out under two conditions, namely the optimized extract and the non-optimized extract. Optimization was carried out using Response Surface Methodology (RSM) on the variables of the extraction process, such as solvent polarity, extraction time, and temperature. The results showed that IC_{50} values for the optimized samples were 20.64 µg/mL and 28.50 µg/mL for the non-optimized samples. Research by Lourith[18] was conducted on mangosteen rinds which was extracted with ethanol (1:50) and deionized water (1:100) and then macerated for 30 and 60 min. The IC_{50} value obtained for the ethanol extract was 6.16 ± 0.01 µg/mL and 11.39 ± 0.03 µg/mL for the aqueous extract. Based on various previous studies, it can be concluded that different mangosteen growing sites, harvest time, extraction method and extraction solvent will give different result in IC_{50} values obtained.

In the CUPRAC method, the percentage of CUPRAC capacity will be determined. A ligand is required to form a copper-ligand complex whose absorbance can be measured. The most commonly used ligand is neocuproin. This method is based on the reduction of Cu^{2+} to Cu^{+} using the reagent Cu^{2+}-neocuproin $(Cu(Nc)_2^{2+})$ as a chromogenic oxidizing agent. An orange Cu^{+}-neocuproin complex is formed with the maximum absorbance value at a wavelength of 450 nm. The greater the absorbance, the greater the percentage of CUPRAC capacity.[13]

In present research of antioxidant activity assay using the CUPRAC method, it can be seen that the leaves sample had highest antioxidant activity of each extraction solvent. In the n-hexane extract, the leaves (L1) had antioxidant activity of 213.060 ± 12.590 mg AAE/g. In the ethyl acetate extract, the leaves (L2) had an antioxidant activity of 357.440 ± 14.363 mg AAE/g. While in the ethanol extract, the leaves (L3) had antioxidant activity of 570.400 ± 78.098 mg AAE/g. It can be seen in Figure 2, the test results for the highest antioxidant activity of each sample were found in the ethanol extract and antioxidant activity by CUPRAC method of various extracts had a different result in the range of 116.360 to 570.400 mg AAE/g.

Based on previous research by Muzykiewicz[19] on the antioxidant activity using the CUPRAC method was carried out on mangosteen rinds that was extracted using an ultrasonic bath with 96% ethanol. The antioxidant activity value was 4.84 ± 0.19 mg Trolox/g RM (raw material). In accordance to the equivalence of ascorbic acid, the results obtained from each ethanol extract can be said to be very different. This can be influenced by differences in the place of growth and age of the plants used during the study as well as differences in the extraction methods used in the study.

In the determination of TPC, reaction that occurs is the reduction of phosphotungstate-phosphomolybdenum in the Folin-Ciocalteu reagent to a blue heteropolymolybdenum so that the reaction results can be measured using UV-visible spectrophotometer at λ 765 nm.[7] The standard solution was reacted with Folin-Ciocalteu reagent and Na_2CO_3 then incubated for 15 min so that the occurred reactions could be optimum. The added of Na_2CO_3 will provide an alkaline atmosphere so that the Folin-Ciocalteu reduction reaction by the hydroxy group on phenol compounds can be occurred.

The TPC in various extract from leaves, branches and rinds had a different result in the range of 10.680 – 49.525 g GAE/100 g extract (Figure 1). The highest concentration between each solvent from each part of the plant was showed by ethanol extract. The highest phenolic content (49.525 g GAE/100 g) was exposed by ethanol leaves extract (L3), while the lowest results (10.680 g GAE/100 g) was given by n-hexane branches extract (B1).

The previous study by Pothitirat[20] on the total phenol content using the young and ripe fruit rinds of mangosteen extracted using 95% ethanol, presented that the TPC was 42.57 ± 0.11 g GAE/100 g in young rinds

and 28.88 ± 0.73 g GAE/100 g in ripe rinds. This result revealed that the more ripe rinds be used gave the lower amount of phenol content. If the study was compared with the ethanol rinds extract, it was shown that the total phenol content obtained didn't differ much. Research on total phenol content by Chaovanalikit[15] was carried out using samples of flesh, outer pericarp and inner pericarp of mangosteen with extraction using acetone and the residue obtained was dissolved in 0.1% HCl. The phenol content was 0.13 ± 20.44 g GAE/100 g for flesh, 2.93 ± 318.10 g GAE/100 g for outer pericarp, and 3.40 ± 321.92 g GAE/100 g for inner pericarp. Zadernowski[21] conducted a study on the total phenol content in exocarp (peel), mesocarp (rind) and pulp (aril) of mangosteen that were extracted 6 times with 80% (v/v) water-methanol. The results obtained were 70.2 ± 5.7 g CE (catechin equivalents)/kg dm (dry matter) in the exocarp, 218.1 ± 18 g CE/kg dm in the mesocarp and 6.4 ± 0.5 g CE/kg dm in the pulp. Research conducted by Zarena[22] on mangosteen rinds extracted using SOXTEC showed total phenol content of 135.9 ± 0.03 mg GAE/g in n-hexane extract and 269.9 ± 0.02 mg GAE/g in ethyl acetate extract. Jaisupa[16] conducted research on mangosteen rinds that was macerated using ethanol with low temperature heating and then concentrated using a rotary evaporator. The total phenol content was 62.15 ± 3.57 mg GAE/g DW (dry weight).

In the determination of TFC, reaction that occurs is the formation of $AlCl_3$ complex with a keto group at C-4 and a hydroxyl group at C-3 or C-5 which is acid-stable. In addition, $AlCl_3$ can form complexes with ortho dihydroxy groups on ring B of flavonoids which are unstable to acids. The complex that obtained can be measured using UV-visible spectrophotometer at λ 415 nm.[8]

The TFC in various extract from leaves, branches, and rinds had a different result in the range of 0.707 – 13.859 g QE/100 g extract (Figure 1). N-hexane extract had the highest concentration between each solvent from each part of the plant. The highest flavonoid content (13.859 g QE/100 g) was given by n-hexane rinds extract (P1) while ethanol rinds extract (P3) showed the lowest value (0.707 g QE/100 g).

Pothitirat[20] also conducted a study on the total flavonoid content using the young and ripe fruit rinds of mangosteen that was extracted using 95% ethanol. The TFC was 2.91 ± 0.09 g QE/100 g in young rinds and 4.08 ± 0.07 g QE/100 g in ripe rinds. This result exposed that the more ripe the fruit rinds be used gave the higher the amount of flavonoid contained. If the study was compared with the ethanol rinds extract, it was showed that the total flavonoid content obtained was quite far. Jaisupa[16] conducted research on mangosteen rinds that was macerated using ethanol with low temperature heating and then concentrated using a rotary evaporator. The total flavonoid content obtained was 260.44 ± 3.03 mg QE/g DW (dry weight). Research on the total flavonoid content was carried out by Ghazemzadeh[17] on the mangosteen rinds with the extraction method using a microwave. The experiment was carried out under two conditions, that was the optimized extract and the non-optimized extract. Optimization was carried out using Response Surface Methodology (RSM) on the variables of the extraction process, such as solvent polarity, extraction time and temperature. The total flavonoid content was 279.19 ± 19.55 mg QE/100 g DM (dry matter) from the optimized extract and 192.5 ± 17.28 mg QE/100 g DM from the non-optimized extract. It can be concluded that the optimization process significantly increased the total flavonoid content. Research by Lourith[18] was conducted on mangosteen rinds which was extracted with ethanol (1:50) and deionized water (1:100) and then macerated for 30 and 60 min. A yellow light ethanol extract was obtained with a total flavonoid content of 2339.48 ± 4.96 mg QE/100 g and a concentrated yellow aqueous extract of 8007.68 ± 2.18 mg QE/100 g. The color difference of the extract was influenced by the flavonoid concentration in the sample. The total flavonoid content in the aqueous extract was much higher than the ethanol extract, it was influenced by the difference in the concentration ratio at the time of extraction and the time used during maceration. Ngawhirunpat[12] conducted a study using mangosteen rinds which was ground and then macerated for 7 days using n-hexane. The total flavonoid content was 6.85 ± 0.43 g EE (epicatechin equivalents)/100 g.

Differences in research results with the results of previous studies can be caused by differences in the origin of mangosteen, harvest time, storage conditions, extraction methods, extraction solvents used, and differences in the method of quantification of total phenolics and flavonoids.[21]

The correlation between total phenolic and flavonoid content in the various extracts of leaves, branches, and rinds of mangosteen on antioxidant activity statistically tested using the Pearson's method. The TPC and TFC were declared to have a contribution if they gave a positive and significant correlation to the antioxidant activity. This indicated that the higher TPC and TFC values gave the higher antioxidant activity of the sample. According to Table 1, TPC and TFC gave a positive and significant correlation to the value of antioxidant activity using the DPPH method, except for the n-hexane leaves extract which showed no significant difference (r = 0.415 for TPC; r = 0.319 for TFC). Meanwhile, TPC and TFC gave a positive and significant correlation to the value of antioxidant activity for all extracts using the CUPRAC method**.** Antioxidant activity with various methods gave positive results on total phenol levels, so it can be said that phenolic compounds are the main contributor that provide antioxidant activity in natural ingredients.[16]

The antioxidant activity provided by phenol group compounds such as flavonoids, phenolic acids, and tannins are influenced by the chemical structure of the phenol group itself. Differences in the structure of phenols and flavonoids can affect differences in antioxidant activity in a sample. The more hydroxyl groups in the aromatic ring and the ortho-diphenol structure, the more antioxidant activity increases.[23] In addition, the antioxidant activity provided by flavonoid compounds are also influenced by the chemical structure of the flavonoid group itself. Flavonoids that have ortho di-OH groups at C-3'-C-4', OH groups at C-3, ketone groups at C-4, double bonds at C-2 and C-3 will provide high antioxidant activity. Meanwhile, glycosylated flavonoids will reduce the number of hydroxy groups in their structure, so that their antioxidant activity will also decrease.[24] Besides being influenced by the amount of phenolic compound content, antioxidant activity could also be influenced by the type of phenolic compound contained in the sample. Analytical procedures such as sample preparation in different ways also significantly affect the antioxidant activity of phenolic compounds.[25]

The correlation between DPPH and CUPRAC methods in the various extracts of leaves, branches and rinds of mangosteen were statistically tested using the Pearson's method. The results in Table 2 showed that there were positive and significant correlation between the two antioxidant activity methods for all extracts. This indicated that DPPH and CUPRAC methods in the present research gave linear results in determining antioxidant activity.

In the present study, determination of flavonoid compounds levels in ethanol rinds extract of mangosteen was using two standards, namely rutin and kaempferol which were known to be present in mangosteen.[26,27] The HPLC chromatogram results of ethanol rinds extract (Figure 3) showed two peaks aligned with the peaks from standard mixture at retention times of 8.119 and 11.829 min, which means there were rutin and kaempferol contained in the extract. From the calculation, it was obtained that the levels of rutin was 0.0327% and kaempferol 0.0049%.

CONCLUSIONS

The antioxidant activity of mangosteen leaves, branch and rinds extracts had antioxidant activity of DPPH in the range of 39.920 – 489.708 mg AAE/g and antioxidant activity of CUPRAC 116.360 – 570.400

mg AAE/g. The highest TPC was given by ethanol leaves extract (L3) (49.525 ± 4.263 g GAE/100 g) and the highest TFC by n-hexane rinds extract (R1) (13.859 ± 1.451 g QE/100 g). The ethanol rinds extract (R3) had the highest antioxidant activity using the DPPH method, while the ethanol leaves extract (L3) had the highest antioxidant activity using the CUPRAC method. Total phenol and flavonoid levels correlated positive and significant with the antioxidant activity using the DPPH and CUPRAC methods, except for the n-hexane leaves extract using the DPPH method. The DPPH and CUPRAC methods gave linear results in determination of the antioxidant activity of mangosteen leaves, branches and rinds extracts. Determination of flavonoid compounds levels showed that the ethanol rinds extract contained rutin 0.0327% and kaempferol 0.0049%. Leaves, branches and rinds of mangosteen were potential to be developed as natural antioxidant sources.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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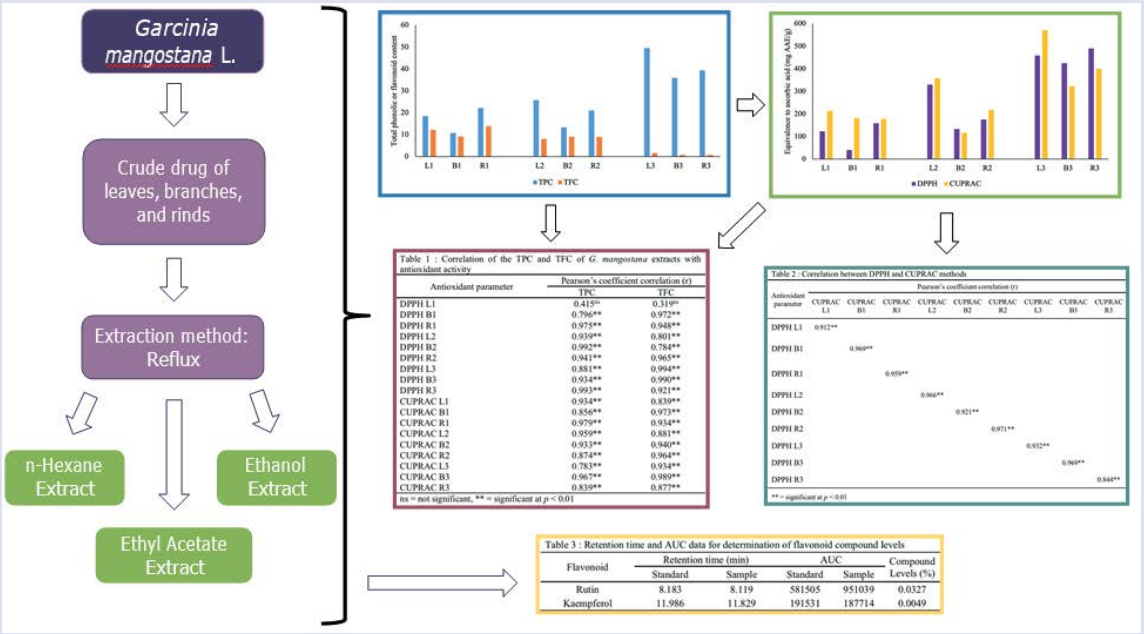
GRAPHICAL ABSTRACT



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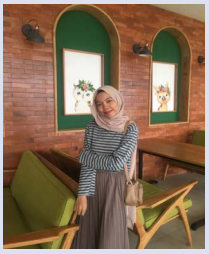

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