Antioxidant and Alpha Glucosidase Inhibition Activity of Kupa (Syzygium Polychepalum Miq.) Cortex

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ABSTRACT

Myrtaceae is one of the families that has been widely studied for its antioxidant activity, and many species belonging to this family have strong activity. Kupa (Syzygium polycephalum), belonging to Myrtaceae family, has not been studied in terms of both chemical and pharmacological effects. The aim of this study was to evaluate the antioxidant and the inhibition activity of alpha glucosidase enzyme, and determine the correlation between the content of the extract and the inhibition activity of alpha glucosidase enzyme. The parameters measured in this research were the total flavonoid content (TFC), total phenolate content (TPC), antioxidant activity, and alpha glucosidase enzyme activity of both the extract and fraction. The extract obtained using reflux method showed the strongest IC_{50} of antioxidant activity, the highest TFC and TPC. While ethyl acetate fractions and ethanol fractions were the fractions that had very strong antioxidant activity, and significantly correlated with IC_{50} of inhibition of alpha glucosidase. According to the results, kupa plant (Syzygium polycephalum Miq) has the potential to be developed as an alternative source of natural antioxidant and diabetic therapy.

Key Words: kupa, Syzygium polycephalum, antioxidant activity, inhibition activity of alpha glucosidase enzyme

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INTRODUCTION

Diabetes is a metabolic disorder that affects the body's ability to produce or use insulin. The main characteristic of diabetes mellitus is an increase in blood glucose level [1,2]. Diabetes prevalence continues to increase every year. By 2015, diabetes in the world reaches 415 million people, and it is estimated to reach 642 million in 2040. Diabetes and its complications are the leading causes of death in many coun
tries. And Indonesia occupies the seventh position in the number of diabetics, with the number of patients as many as 10 million. Up to 2015, 184.985 people in Indonesia have died from diabetes [3].

There are many factors that can lead to diabetes, one of which is oxidative stress. Oxidative stress plays an important role in the development of diabetes and its complications [4], which results from an imbalance between the number of radical molecules and antioxidant compounds in the body. Antioxidants are substances that can inhibit or prevent damage or destruction due to oxidation [5]. Basically, the body has endogenous antioxidants, such as catalase enzymes, glutathione peroxidase, superoxide dismutase and glutathione S-transferase. But, if free radicals that enter the body continue to increase, then the body needs antioxidants from outside in the form of exogenous antioxidants [6]. Exogenous antioxidants

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can be found in abundance in both vegetables and fruits [7]. The antioxidant effects of these exogenous sources are mainly due to the presence of phenol compounds such as flavonoids, phenolic acids, and tannins [8,9,10].

The plant that has been widely evaluated for its antioxidant activity belonged to Myrtaceae family [11,12,13]. While kupa (Syzygium polycephalum Miq.), belonging to the Myrtaceae family, has not been studied in terms of both chemical and pharmacological effects. But based on the preliminary studies, kupa cortex showed the strongest antioxidant activity, with the IC_{50} value for ethanol extract of kupa cortex 12,58 µg / mL, compared to the fruit, leaves, and wood [14].

In Indonesia, people recognize the kupa plant as a fruit plant. The kupa fruit is consumed as traditional salad, the leaves edible as fresh vegetables, and the wood part can be used for building constructions [15], while the cortex is used for the traditional treatment of dysentry [16]. However, in the Philippines, this plant is used to lower high blood pressure and high cholesterol level. The cortex part is used for the treatment of dysentry [17].

Kupa has been known to have some pharmacological activities such as antidiabetes, antifungi and antioxidants. Kupa ethanol extract has the activity as antihiperglikemia with the mechanism of inhibiting alpha glucosidase enzyme with IC_{50} value = 37,74 ppm [18]. Some fractions of kupa lignum had antifungal activity [19]. The ethanol extract of kupa cortex had antioxidant activity with IC_{50} 12,584 µg / mL and vitamin C with IC_{50} 8,946 µg / mL as a standard drug [14].

The aim of this study was to evaluate the antioxidant and the inhibition activity of alpha glucosidase enzyme, and determine the correlation between the content of the extract and the inhibition activity of alpha glucosidase enzyme.

MATERIAL AND METHODS

Materials

DPPH (2,2-diphenyl-1-picrylhydrazyl), gallic acid, quercetin, beta carotene purchased from Sigma-Aldrich (MO, USA), kupa cortex (Syzygium polycephalum), ethanol were the materials used in this study. All the reagents were of analytical grades.

Extraction

Kupa (Syzygium polycephalum) cortex was obtained from Banjaran area, Bandung, West Java, Indonesia. Fresh plants were dried at 60-70°C, and then ground into small pieces. The plant identification and authentication were performed by Biological Department, Padjajaran University. Phytochemical screening included examination of alkaloids, flavonoids, saponins, quinones, phenolic, tannins, and steroids / triterpenoids compound. The extraction method was macerated using 95% ethanol, and then concentrated using rotary evaporator.

Determination of Total Flavonoid Content (TFC)

The samples and standards were dissolved in methanol added 2% AlCl3 in 95% ethanol in a 1: 1 volume ratio, and incubated for 1 hour. The absorbance was measured at a wavelength of 420 nm using an ultraviolet-visible spectrophotometer. Flavonoid content was calculated against the calibration curve with quercetine as a standard flavonoid compound with a range concentration of 8 to 20 µg / mL in methanol [20,21].

Determination of Total Phenolic Content (TPC)

The determination of phenol content in the extract was analyzed by using folin-ciocalteu reagent. The solution was prepared by dissolving the extract with methanol-water (1: 1), then filtered using Whatman paper no 4. As much as 0.5 mL of the sample extract was added with 5 ml of Folin-ciocalteu reagent (previously diluted with water 1: 10), and then incubated for 15 minutes. The standard phenolic compound was gallic acid. The standard underwent the same treatment as the extract. The extracts and standards were measured at a wavelength of 765 nm. The total phenolic content was calculated as gallic acid (GAE mg) from the calibration curve [22].

Evaluation of Antioxidant Activity

Evaluation of antioxidant activity was performed on ethanol extract. The reagents used are DPPH (1,1-diphenyl-2-picrylhydrazyl), and measured by spectrophotometry. The samples and standards dissolved in methanol were added to DPPH stock solution (ratio 1: 1), and then incubated for 30 min at room temperature using a dark aluminum foil coated container in a closed state. The absorption was measured at 516 nm wavelength. The percent of decrease in DPPH absorbance was calculated using the formula:

$I(\%) = (Ao - As)/ Ao \times 100$

I: percent decrease in absorbance of DPPH

Ao: absorbance of control DPPH solution

As: absorbance of sample solution after DPPH is added

The correlation between the percentage of decrease of DPPH absorbance in stock solution was determined. The effective concentration value was calculated from the regression equation obtained by entering the 50% inhibition as the dependent variable [21,23,24].

In Vitro Alpha Glucosidase Inhibitory Activity Evaluation

The inhibitory activity of alpha glucosidase was carried out by using purified and commercial alpha glucosidase enzymes, a measurement based on methods reported by [26]. Alpha glucosidase enzyme of 100 µL (0.45 units / mL) was added with 25 µL glutathione (1.0 mg / mL), 100 µL phosphate buffer 0.67 mM (pH 6.8), and 725 µL extract with various concentrations. The mixture was stirred and incubated at room temperature for 10 min, and then 50 µL PNPG (0.01 M) was added. The absorbance was measured by a spectrophotometer at 405 nm λ every 30 seconds for 5 minutes. The inhibitory activity was calculated by comparing the absorbance of the extract to the acarbose as a standard drug [25].

RESULTS AND DISCUSSION

The antioxidant activity, flavonoid, phenolic and carotenoid content of kupa cortex extracts from different extraction methods are presented in Table 1. The method used in this study was maceration and reflux with ethanol 96% as a solvent.

Table 1. Antioxidant activity and flavonoid, phenolic and carotenoid content of Syzygium polycephalum

Extraction method	Yielded extract (%)	Specific gravity (g/ml)	Antioxidant activity[1]	Total flavonoid content[2]	Total phenolic content[3]	Total carotenoid content[4]
Maceration (ME)	2.2	0.819	24. 248	0.889±0.009	14.493±0.537	0.336±0.221
Reflux (RE)	11.89	0.836	19.325	1.229±0.063	17.453±0.849	0.187±0.009

[1]IC_{50} (µg/ml), [2]mg quercetin equivalent/ 100 mg extract, [3]mg GAE equivalent/ 100 mg extract, [4]mg carotenoid equivalent/ 100 mg extract

The extract obtained from the reflux method gave a greater amount of extract than the maceration method, and it was comparable to higher levels of flavonoid and phenolic content of the reflux extract. The reflux is a hot extraction, and heating can increase the amount of extracted compounds. The results were inversely proportional to total carotenoid content,the extract of maceration had higher carotenoid content than the reflux extract, ie, 0.336 ± 0.221 and 0.187 ± 0.009 mg carotenoid equivalent / 100 mg extract, respectively. This might be due to unstable carotenoid compounds to heat, so the use of heat would lead to the decomposition of carotenoids and lessen the extract's total carotenoid content.

Reflux extract had stronger antioxidant activity than maceration extract with IC_{50} values 19,325 and 24.248 µg / ml, respectively. This might be due to the point that reflux extract had higher total flavonoids and phenolic content. According to the phytochemical screening, the extract of kupa cortex contained flavonoid, tannin, phenolic compound, quinone, saponin, and steroid/ triterpenoud compound. Phenolic compound is distributed widely in plants, and has antioxidant activity. Phenolic compound, flavonoid and terpenoids had activity by inhibiting alpha glucosidase enzyme.

IC50 of DDPH scavenging activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) is a soluble free radical in methanol, and ethanol has a maximum absorption at 515-520 nm. DDPH free radicals are characterized by purple, and will turn yellow when stabilized by antioxidants.

The evaluation procedure followed Blois [23], DDPH was dissolved in methanol and the obtained maximum absorption was at 516 nm. The samples and standards were dissolved in methanol, extracts and fractions with various concentrations mixed with 60 µg/ml DPPH (1: 1), after incubation for 30 minutes, the absorbance was measured at 516 nm using Shimadzu® UV-1800 UV-Vis spectrophotometer. As standard drug used ascorbic acid, the sample and standard measurements were done three times. Antioxidant activity was determined by calculating the percentage of DPPH inhibition and determination of IC_{50} from the obtained calibration curve [24].

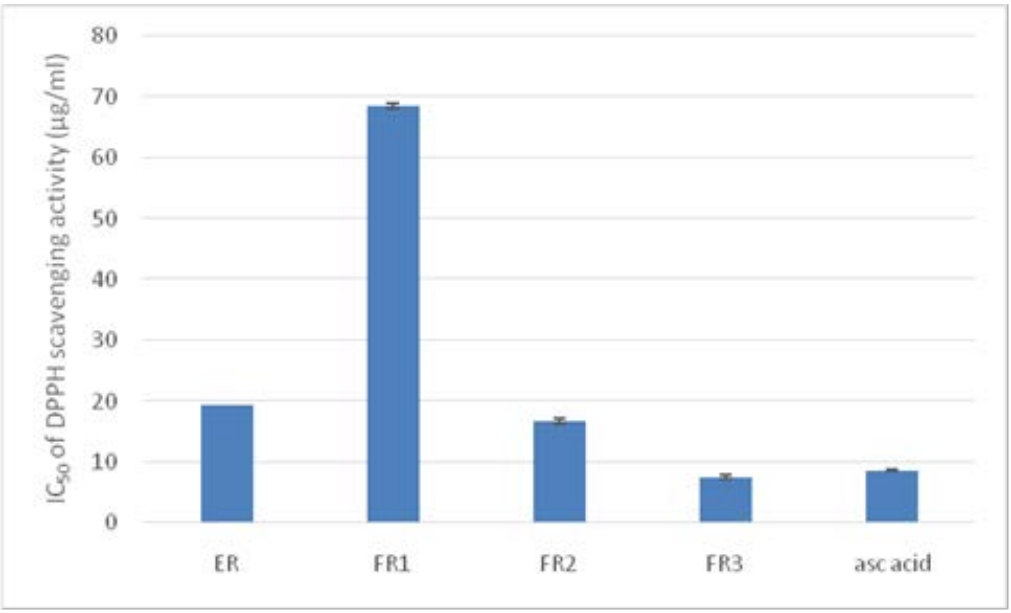

Fig 1. IC_{50} of DPPH scavenging activities of extract and fraction kupa. ER=reflux extract, FR1=n-hexane fraction, FR2=ethyl acetate fraction, FR3=ethanol fraction.

DPPH inhibition activity evaluation results could be seen in Fig 1. Inhibition concentration 50 (IC_{50}) was a concentration that could decrease the activity of 50% of DPPH. The smaller the value of IC_{50}, the stronger the antioxidant activity.

A compound with IC_{50} below 50 µg / ml was classified into very active antioxidant category. Fig 1 showed that reflux ethanol extract (ER) with IC_{50} value of 19.325 µg / ml, ethyl acetate fraction (FR2) with IC_{50} value of 16.728 µg/ml, ethanol fraction (FR3) with IC_{50} value of 7.567 µg/ml, and ascorbic acid as a standard drug with IC_{50} value of 8,698 µg / ml were included in this category. The compounds in the extract were separated according to their polarity by liquid-liquid extraction, so that each fraction had different antioxidant activity. Flavonoid and phenolic compounds had good solubility in ethyl acetate and ethanol, so these two fractions had a stronger antioxidant activity compared to n-hexane fraction. And according to the results, the extract had stronger antioxidant activity compared to the ascorbic acid as a standard drug.

Total Flavonoid content (TFC)

The flavonoid level of the extract and fraction were calculated using the calibration curve equation of quercetin y = 0.032x + 0.080, R^2 = 0.998. TFC of reflux ethanol extract (ER) was 1.229 mg QE / 100 mg extract. The extract was fractionated with liquid-liquid extraction, so that the compounds in the extract will be separated according to their polarity. The results can be seen in Fig 2. Flavonoid level for the fraction of n-hexane, ethyl acetate and ethanol were 0.517, 1.304, 2.987 mg QE / 100 mg fraction, respectively. Ethanol had the highest concentration of flavonoid compared to the other fractions. It was because of the reason that many of the flavonoids had a high polarity, so it was more soluble in ethanol rather than in n-hexane or ethyl acetate.

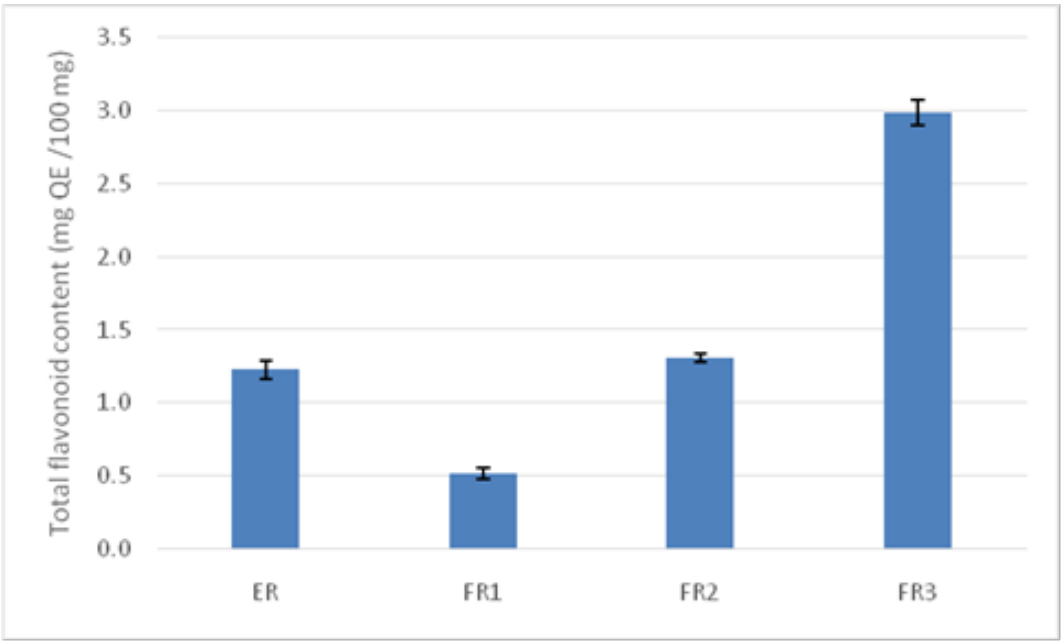

Fig 2. Total flavonoid content of extract and fraction kupa. ER=reflux extract, FR1=n-hexane fraction, FR2=ethyl acetate fraction, FR3=ethanol fraction.

Total phenolic content (TPC)

The phenolic compound level of the extract and fraction were measured using calibration curves of gallic acid y = 0.005x + 0.005, R^2 = 0.995. The TPC of extract ethanol reflux (ER) was 17.453 mg GAE / 100 mg extract. The extract was fractionated using liquid-liquid extraction, so that the compounds in the extract will be separated according to the polarity. The results can be seen in Fig 3. The TPC for n-hexane, ethyl acetate, and ethanol fraction were 12.327; 22.707; 27.480 mg QE / 100 mg fraction, respectively.

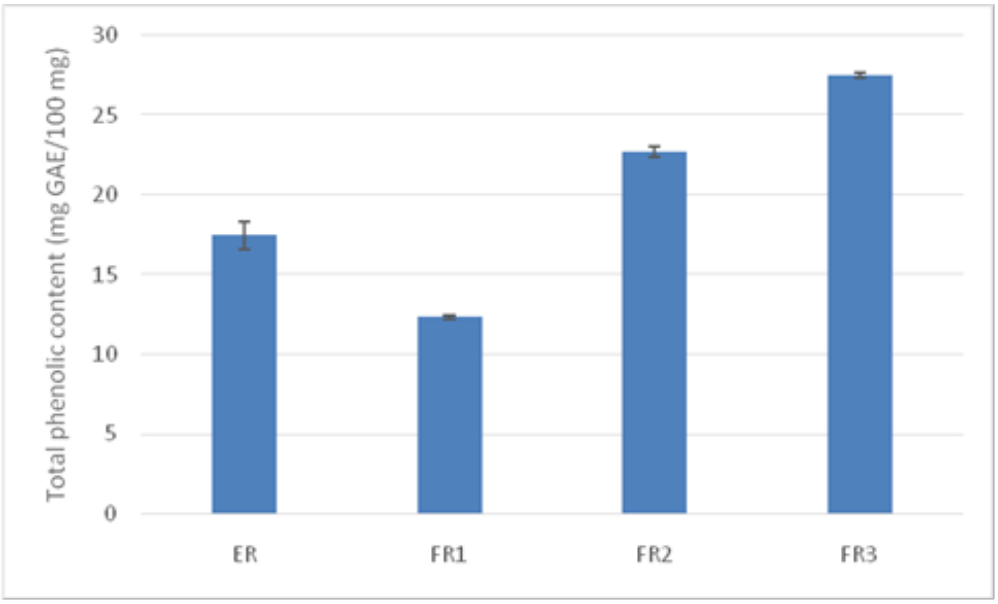

Fig 3. Total phenolic content of extract and fraction kupa. ER=reflux extract, FR1=n-hexane fraction, FR2=ethyl acetate fraction, FR3=ethanol fraction.

The optimization of enzyme concentration was performed to determine the optimal concentration of the enzyme. The enzyme concentrations were 0.3; 0.4; 0.5; and 0.6 U/mL with pNPG substrate concentration (p-nitrophenyl-α-D-glucopyranoside) 20 mM. Based on the optimization result, the enzyme concentration of 0.5 U/mL gave the highest absorbance, which was 0.811 and still in optimal absorbance range according to Lambert-Beer. The higher the absorbance, the more products were formed, so that the enzyme concentration and the substrate was said to be optimal. Furthermore, the enzyme concentration used was 0.5 U / mL and the substrate concentration used was 20 mM.

The α-glucosidase enzyme inhibiting activity was performed on ethanol extract and the fractions obtained from liquid-liquid extraction, which were n-hexane fraction, ethyl acetate fraction, and ethanol fraction; and acarbose as standard drug. Acarbose was an α-glucosidase-inhibiting agent commonly used in the treatment of type 2 diabetes mellitus. The principle of reaction occurring between the enzyme and the substrate was the hydrolysis of the pNPG substrate by the α-glucosidase enzyme into glucose and p-nitrofenol which produced the yellow color. Inhibitory activity was measured by absorbance of yellow p-nitrofenol at a wavelength of 405 nm.

The result showed that ethanol fraction had the strongest enzyme inhibiting activity with IC_{50} 2.97 µg / mL, while acarbose had IC_{50} value of 9.68 µg / mL. Many compounds of natural materials such as terpenoids, flavonoids, and phenolics have potential as antidiabetes. Some flavonoids, polyphenols, and sugar derivatives effectively inhibit the activity of the α-glucosidase enzyme.

Table 2. Pearson's correlation coefficient of total flavonoid compound (TFC), total phenolic compound (TPC), IC_{50} of DPPH and IC_{50} of inhibition alpha glucosidase

	IC_{50} inhibition alpha glucosidase	TFC	TPC
IC_{50} DPPH ER	-0.98	-0.746	0.95
IC_{50} DPPH FR1	-0.631	0.568	-0.914
IC_{50} DPPH FR2	0.997*	-0.434	0.487
IC_{50} DPPH FR3	0.998*	0.875	0.539

*Correlations significant at the 0.05 level

The antioxidant activity of the hexane fraction (FR1) was positively correlated with total phenolic compound (TFC, r = 0.568), and negative correlation to IC_{50} inhibition alpha glucosidase (r = -0.631), and negatively high correlation with TPC (r = -0.914). The antioxidant activity of ethyl acetate fraction (FR2) was significantly correlated with IC_{50} inhibition alpha glucosidase (r = 0.997) and positively correlated to TPC (r = 0.487). The antioxidant activity of the ethanol fraction (FR3) was significantly correlated with IC50 inhibition alpha glucosidase (r = 0.998), and positively correlated with TFC and TPC (r = 0.875, r = 0.539, respectively) (Table 2).

CONCLUSSION

Reflux extraction provided the extract with the strongest IC_{50} antioxidant activity, and the highest TFC and TPC. Ethyl acetate fractions and ethanol fractions were the fractions that had very strong antioxidant activity, and significantly were correlated with IC_{50} inhibition alpha glucosidase. According to these results, kupa plant (Syzygium polycephaum Miq) has the potential to be developed as an alternative source of natural antioxidant and diabetic therapy.

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