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THE FREE RADICAL SCAVENGING AND ANTI-HYPERGLYCEMIC ACTIVITIES OF VARIOUS GAMBIERS AVAILABLE IN INDONESIAN MARKET

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Abstract

Gambier (*Uncaria gambier*) is known to have antioxidant properties, and some studies have attributed it to the presence of polyphenols such as catechin. The objective of this study is to investigate the potential of various gambiers available in Indonesian market as a scavenger of reactive free radicals and evaluate its anti-hyperglycemic activity as α-glucosidase inhibitor. Isolation of catechin was done by extraction method with technical grade of ethyl acetate as solvent. Analysis of catechin in the dried gambier extract was carried out with TLC method. The molecular weight and content of catechin of dried gambier extract was determined by analyzing its mass spectra and spectrophotometer, respectively. The free radical scavenging activity of catechin of the resultant extracts was measured by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) as stable free radical compound. The anti-hyperglycemic activity of catechin of ethyl acetate extracts was analyzed as α-glucosidase inhibitor. The result showed that various gambiers available in the market are very active as antioxidant, indicated by IC_{50} of catechin of ethyl acetate extracts which were 4.6 to 18.2 μg/mL for DPPH inhibition. The IC_{50} for α-glucosidase inhibition is ranged from 40.45 to 52.43 μg/mL, so they can be classified as anti-diabetic.

Keywords: α-glucosidase, catechin, DPPH, extraction, Uncaria gambier

1. Introduction

Diabetes or also known diabetes mellitus (DM) is the most chronic metabolic disorder and is characterized by high blood glucose levels called hyperglycemia [1]. At the present time, it is estimated that 220 million people worldwide have DM, and that this will increase to 300 million by 2025. Globally, the percentage of type 2 DM (non-insulin dependent diabetes (NIDDM)) is greater than 90%, and the rest is type 1 DM (insulin-dependent diabetes mellitus (IDDM)) [2].

The early symptoms of metabolic disorder that occurs in patient type 2 DM is the quick increase of post-meal blood sugar levels called postprandial hyperglycemia. One of the therapeutic approaches to decrease postprandial hyperglycemia is to retard absorption of glucose by the inhibition of carbohydrate hydrolyzing enzymes, for example α-amylase, α-glucosidase, sucrose, and maltose. In DM patients, inhibition of these enzymes restrains glucose absorption and decrease the postprandial hyperglycemia [3-4].

Although acarbose, miglitol, voglibose, and nojirimycin are known to be potent inhibitors of α-glucosidase, further screening for α-glucosidase inhibitors from natural sources such as plants and microorganism will help in reducing overdependence on synthetic drugs. A number of anti-hyperglycemic agents have been found in plants as new therapeutic drugs for type 2 DM. These plants are typically rich in phenolic compounds, which are known to interact with proteins and can inhibit enzymatic activity of α-glucosidase and α-amylase [5-6].

Gambier plant (*Uncaria gambier*) is classified into the family of *Rubiaceae*, from the genus of *Uncaria* [7]. This gambier species is commonly found in tropical regions such as Indonesia and used as traditional medicine for burned skin, digestive problem, fever, headache, and utilized as antibacterial or antifungi [8-12]. Some researchers have published the utilization of poliphenolic compounds of gambier. catechin, as an example, which has been reported to have antioxidant activity and antibacterial against gram positive bacteria *Staphylococcus aureus*, *Bacillus subtilis*, and *Streptococcus mutan* [13-16].

In Indonesia, gambier is commonly chewed by old people together with betel leaves. Commercial gambier is produced by boiling gambier leaves and branches, continued with compressing, and drying. The main phenolic compound of gambier is catechin. In trade world, a parameter used to express the quality of gambier is catechin content. The minimal catechin content of first, second, and third quality of gambier are 40%, 30%, and 20%, respectively [17]. Even though gambier is rich in polyphenols, it is not used traditionally in treating DM, so it is necessary to study its potential of gambier as a α-glucosidase inhibitor.

In the present study, we investigated the free radical scavenging and anti-hyperglycemic activities potential in commercial gambier sold in a local Indonesian market. The free radical scavenging activity analysis was performed by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) as sources of free radical [18]. The anti-hyperglycemic activity analysis was performed by using α-glucosidase enzyme and *p*-nitrophenyl α-D-glucopyranoside as a substrate (Kim Yong-Mu's method) [6].

2. Experiment

Materials and equipments. Materials used in this experiment were crude gambiers from Payakumbuh, Padangpanjang, Lampung, and commercial ones from Jakarta, packed, labeled, and sold as SP, Super, and Spr brands. The technical grade solvent was ethyl acetate. Some analytical grade chemicals were used for molecular weight, DPPH radical scavenging and α-glucosidase inhibitory activities analysis.

The equipments used in this experiment were one extraction unit, one evaporation unit, and one set of catechin identification unit. Thin layer chromatography (TLC) was carried out using precoated Silica gel plates (Merck Kieselgel 60F 254, 0.25 mm). Mass spectra (MS) or molecular weight were obtained with *liquid chromatography-mass spectroscopy* (LC-MS), mariner biospectrometry spectrometer using electrospray ionization (ESI), system and positive ion mode. DPPH radical scavenging and α-glucosidase inhibitory activities analysis were evaluated by using spectrophotometer Spechtronic Hitachi U2000.

Isolation of catechin. Isolation of catechin from gambier was done through extraction. Approximately 500 g of gambier powder with particle size of 100 mesh was put into an extractor unit with loading capacity of 1 kg, and 1.5 litres of technical grade of ethyl acetate was added. Solvent rich of catechin was then evaporated using vacuum rotary evaporator to obtain dried extract. The weight and the *rendement* of the dried extract were then evaluated. The catechin content of gambier was the *rendement* of extraction process which was formulated from the weight (dry basis) of extracted material divided by the weight of crude gambier, multiplied by 100%.

TLC, MW, and catechin content analysis. The ethyl acetate extract was analyzed using thin layer chromatography (TLC) and then compared to standard catechin. The mass or molecular weight (MW) analysis was conducted by using LC-MS, while the analysis of catechin content was done using spectrophotometry method according to SNI 01-3391-2000 [17].

The free radical (DPPH) scavenging activity analysis. The DPPH radical scavenging activity analysis was evaluated by using spectrophotometer [18]. Crude gambier and ethyl acetate extract of gambier were dissolved in methanol to obtain final concentration of 10, 50, 100, and 200 μg/mL, in order to find the IC_{50} of the tested solutions 10-200 μg/mL, while ascorbic acid (vitamin C), as standard, was dissolved in methanol with final concentration of 10-25 μg/mL. Sample solution was then reacted with 1 mM DPPH in methanol with total volume of 10-25 mL. The blank was 1 mM DPPH in 2.5 mM of methanol. Incubation was done at 37 °C for 30 minutes and the absorbance was read at 517 nm. The percent inhibition was calculated as follows: % inhibition = [(C-S)/C] x 100%, where C is absorbance of the blank and S is the sample absorbance. The IC_{50} was calculated as the concentration that caused 50% inhibition of DPPH.

The anti-hyperglycemic activity analysis. Inhibition analysis on α-glucosidase enzyme was performed to evaluate the anti-hyperglycemic activity of the samples. α-Glucosidase enzyme solution was dissolved in phosphate-buffer solution (ph 7) containing 200 mg albumin serum. Before its application, 1 mL of the enzyme solution was diluted 25 times with the buffer solution. The reaction mixture consisting of 250 μL of 20 mM *p*-nitrophenyl α-D-glucopyranose as the substrate, 490 μL of 100 mM phosphate buffer (pH 7) and 10 μL of the extract dissolved in DMSO were prepared. The samples concentrations for activity evaluation were 3.125, 6.25, 12.5, and 25 μg/mL. The reaction mixture then was water bath incubated at 37 °C for 5 min. About 250 μL the enzyme solution was added, and kept the solution incubated for 15 min. The enzyme reaction was stopped by addition of 1000 μL, 200 mM sodium carbonate solution. The resulted *p*-nitrophenol from the reaction was measured at 400 nm. Standard solutions of same concentrations were also prepared using quercetin. The percent inhibition was calculated as follows: % inhibition = [(C-S)/C] x 100%, where C is absorbance of the blank (DMSO) and S is the sample absorbance. The IC_{50} was calculated as the concentration that caused 50% inhibition of enzyme activity.

3. Results and Discussion

Although gambier is one of Indonesian export commodities, until now it is exported as crude extract with low selling price. In contrast, refined gambier catechin extract is traded with high selling price. We had a concept to add the economic value of gambier through an appropriate technology, which is inexpensive, simple, and easily implemented in local communities, thus in this research, the isolation of catechin from gambier was done through extraction using technical grade of ethyl acetate as its solvent. The *rendement* of extracted material obtained from crude gambier sample from Payakumbuh was the highest (41.8%), commercial gambier Spr Brand was 35.71%, while the others were about 20 %. According to the catechin content presented by the ethyl acetate extract, the crude gambier from Payakumbuh was classified into first quality; Spr was second quality, while the others were third quality. The catechin content that was calculated by rendement of catechin extraction from gambier is presented in Figure 1.

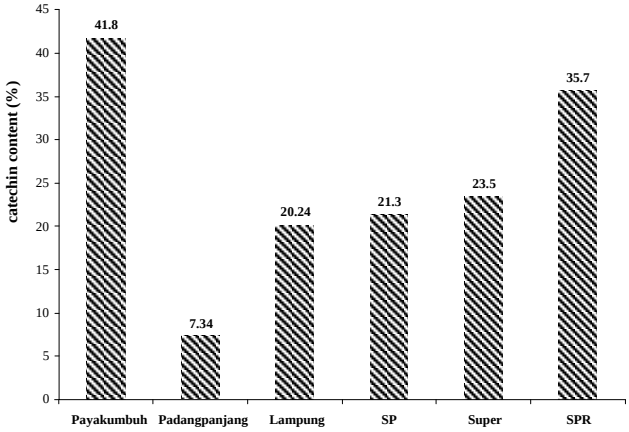

Figure 1. Catechin Content of Crude Gambier

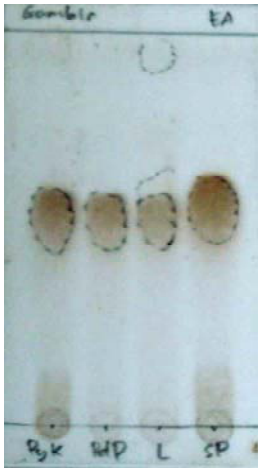

Figure 2. TLC Analysis Result of Catechin of Ethyl Acetate Extract

In order to check the existence of catechin in the ethyl acetate extract, a preliminary analysis was done using TLC, and it was then compared to the standard catechin. The TLC analysis of catechin showed that ethyl acetate extract of the sample gambiers contained catechin, which was pointed by a spot with Rf value of 0.56 equivalent to that of the spot of standard catechin. The result of TLC analysis is presented in Figure 2.

The molecular weight of catechin as the result of ethyl acetate extract was determined by analyzing its mass spectra of LC-MS's chromatogram. The chromatogram of ethyl acetate extracts illustrated 2 peaks: the retention time of the dominant peak was about 1.5 minutes, area under curve (AUC) of 45789.7; and molecular weight of 290.27 was recognized as catechin, as the molecular weight of catechin according to literature was 290.25. The second peak at retention time of 1.2 minutes and AUC of 3996.4 most likely was impurity. The molecular weight (MW) of ethyl acetate extracts are presented in Table 1.

The purity of catechin of the resultant ethyl acetate extract was analyzed by evaluating of catechin content of the resultant ethyl acetate extract using spectrophotometry method, according to SNI 01-3391-2000 [17]. The results are presented in Table 1. According to the analysis of catechin content in ethyl acetate extracts of gambier samples, it could be concluded that the purity of the resultant catechin was relatively high, indicating the successfulness of the extraction process.

The free radical scavenging activity analysis was performed by using DPPH as sources of free radical and was done in various concentration of ethyl acetate extracts solution, 10-200 μg/mL, in order to find the IC_{50} of the tested solutions. The main character of the antioxidant compound or extract is its ability to capture free radical, so that the extract is active against DPPH, so the extract is active as an antioxidant. An extract is categorized as active as antioxidant if its IC_{50} is less than 100 μg/mL. The DPPH is a source of free radical, which captured hydrogen from catechin as antioxidant of ethyl acetate extract, so as DPPH (1,1-diphenyl-2-picrylhydrazyl) turned from purple to yellow, it indicated

Table 1. Molecular Weight (MW) and Purity of Catechin of Ethyl Acetate Extract

Gambier Samples	Retention Time (minute)	Molecular Weight (MW)	Purity of Catechin (%)
Payakumbuh	1.51	290.27	93.94
Lampung	1.57	290.25	91.97
Padangpanjang	1.48	290.28	97.99
SP	1.52	290.28	94.09
Super	1.52	290.27	93.32
Spr	1.57	290.25	97.12

the conversion into 1,1-difenil-2-pikrilhidrazin [18]. This DPPH method used ascorbic acid (vitamin C) as standard, so we know if the tested solutions had the same, less, or higher activity compared to the standard material.

The antioxidant activity analysis showed that crude gambier was an active antioxidant as it had IC_{50} ranged from 10.75 to 40.98 μg/mL. The catechin compound from ethyl acetate extracts showed higher antioxidant activity as they had IC_{50} ranged from 4.55 to 18.23 μg/mL. With IC_{50} less than 50 μg/mL, both crude gambier and ethyl acetate extracts of gambier were categorized as active antioxidants. Besides, catechin in three ethyl aceteate extracts (Payakumbuh, Padangpanjang and Lampung) gave higher antioxidant activity about 2.1 up to 2.4 times than vitamin C, where IC_{50} of vitamin C was 10.97 μg/mL. The antihyper-glycemic activity of ethyl acetate extracts as α-glucosidase inhibitor analysis was evaluated by using α-glucosidase enzyme and *p*-nitrophenyl α-D-glucopyranoside as a substrate. α-Glucosidase enzyme will hydrolyse *p*-nitrophenyl α-D-glucopyranoside become *p*-nitrophenol and glucose. Enzyme activity was measured based on *p*-nitrophenol absorbance result in yellow colour [6].

An extract can be categorized as very active α-glucosidase inhibitor if its IC_{50} is less than 25 μg/mL, active if it is $25 \leq IC_{50} \leq 50$, less active if it is $50 \leq IC_{50} \leq 100$, and not active if its IC_{50} is more than 100 μg/mL [6]. The result of the anti-hyperglycemic activity evaluation showed that crude gambiers and ethyl acetate extracts containing catechin have inhibition effect on α-glucosidase enzyme activity, which means it can inhibit the hydrolysis of carbohydrates into glucose thus preventing the occurrence of postprandial hyperglycemia. Therefore, the extract has potential as therapeutic drugs for type 2 DM. The inhibition of the α-glucosidase enzyme activity restrains glucose absorption and decreases the postprandial hyperglycemia [3]. Crude gambier has moderate α-glucosidase inhibitory activity with IC_{50} from 66.22 to 89.87 μg/mL. The resultant ethyl acetate extracts are moderate and higher than crude gambier with IC_{50} from 40.65 to 52.43 μg/mL, while IC_{50} for α-glucosidase inhibition of standard quercetin was 4.08 μg/mL. Consequently, further experiment on the synthesis of catechin derivative compounds with improved activities α-glucosidase inhibitor needs to be done. In addition, the other polyphenols contained in gambier are possible to have α-glucosidase inhibitory activity higher than catechin, so the extraction of the other polyphenols by using other solvents such as ethanol needs to be done.

The results DPPH scavenging and α-glucosidase inhibition activities of crude and ethyl acetate extract of gambiers analysis are given in Table 2, while the reaction of DPPH of catechin as antioxidant compound is presented in Figure 3.

Table 2. The DPPH and α-Glucosidase Inhibition Activities

Sample	IC_{50} (μg/mL)	
	DPPH	α-Glucosidase
Payakumbuh Crude Gambier	10.75	83.47
Ethyl Acetate Extract of Payakumbuh Gambier	4.88	47.57
Padangpanjang Crude Gambier	40.98	89.87
Ethyl Acetate Extract of Padangpanjang Gambier	5.10	47.47
Lampung Crude Gambier	15.82	75.31
Ethyl Acetate Extract of Lampung Gambier	4.55	48.00
Commercial Gambier SP Brand	28.46	66.22
Ethyl Acetate Extract of Gambier SP Brand	18.22	52.43
Commercial Gambier Super Brand	22.13	70.47
Ehtyl Acetate Extract of Gambier Super Brand	14.71	45.77
Commercial Gambier Spr Brand	30.61	69.12
Ethyl Acetate Extract of Gambier Spr Brand	19.82	40.65
Ascorbic acid (Vitamin C)	10.97	-
Quercetin	-	4.08

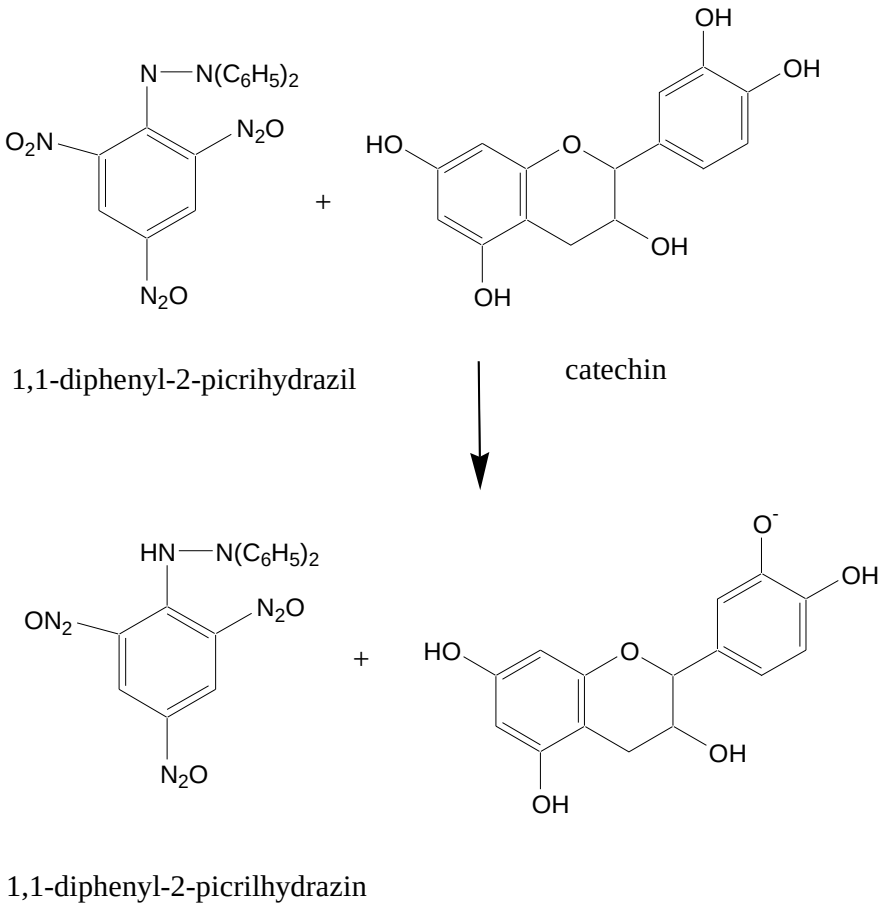

Figure 3. The Reaction of DPPH Free Radical Scavenging of Catechin Compound

4. Conclusion

The mayor bioactive compound of gambier is catechin. According to the catechin content that was calculated from the *rendement* of the extraction catechin from various crude and commercial gambier samples in local Indonesian market, most of the gambier samples were third quality with about 20% catechin content. According to the catechin content of ethyl acetate extracts, which was above 90%, it could be concluded that the purity of the resultant catechin was relatively high.

The crude and ethyl acetate extract of commercial gambier samples have high ability to scavenge reactive free radicals produced by DPPH; hence they have high antioxidant activity. The IC_{50} of catechin that was extracted from various gambier samples in local Indonesian market, ranged from 4.55 to 18.2 μg/mL, was categorized into a very active antioxidant. The antioxidant activity of three ethyl acetate extracts from Payakumbuh, Padangpanjang, and Lampung gambier samples were higher, about 2.1 to 2.4 times of vitamin C.

The crude and resultant ethyl acetate extracts containing catechin have inhibition effect on α-glucosidase enzyme activity, which means they can prevent the occurrence of postprandial hyperglycemia. The crude and ethyl acetate extract of commercial gambier samples have moderate α-glucosidase inhibitory activity with IC_{50} from 40.65 to 89.87 μg/mL, so gambier can be developed as new therapeutic drugs for type 2 DM. Catechin has moderate α-glucosidase inhibitory activity, so further experiment on the synthesis of catechin derivative compounds with improved activities α-glucosidase inhibitor needs to be done.

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