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Free Radical Scavenging Activity and Antihyperglycemic Potency of Various Gambier Collected from Several Indonesian Markets

(Akivitas Penangkapan Radikal Bebas dan Potensi Antihiperglikemik Beberapa Gambir dari Beberapa Pasar Indonesia)

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Abstract: Gambier (*Uncaria gambier*) is known to have antioxidant properties and some studies attributed to the presence of polyphenols. The objective of this study was to investigate the potential of various gambier available in Indonesian market as free radical scavenger and to evaluate its antihyperglycemic potency as α-glucosidase inhibitor. Isolation of catechin was carried out by extraction with distilled ethyl acetate as solvent. Analysis of catechin in the gambier dried extracts were carried out by TLC method. The molecular weight and catechin content of gambier dried extracts were determined by analyzing mass spectra and spectrophotometry, respectively. The free radical scavenging activity of extracts were measured by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) as stable free radical compound. The antihyperglycemic potency of gambier dried extracts were assayed, as α-glucosidase inhibitor. The results showed that various gambier available in the market were very active as antioxidant, indicated by the IC_{50} values of gambier dried extracts were 4.6 to 18.2 μg/mL . Whilst the IC_{50} for α-glucosidase inhibition varies from 40.45 to 52.43 μg/mL, and classified as a moderate antidiabetic.

Keywords: *Uncaria gambier*, antioxidant, antihyperglycemic, extract, α-glucosidase.

Abstrak: Gambir (*Uncaria gambier*) telah diketahui mempunyai aktivitas antioksidan karena mengandung polifenol. Penelitian ini bertujuan untuk mengetahui aktivitas penangkapan radikal bebas dan potensi antihiperglikemik sebagai inhibitor α-glukosidase dari gambir yang dikoleksi dari beberapa pasar Indonesia. Isolasi katekin dilakukan dengan cara ekstraksi dengan menggunakan pelarut etil asetat. Analisis awal katekin yang terkandung dalam ekstrak kering gambir dilakukan dengan KLT. Evaluasi berat molekul dan kandungan katekin pada ekstrak kering gambir dilakukan masing-masing dengan spektroskopi massa dan spektrofotometer. Aktivitas penangkapan radikal bebas diukur dengan menggunakan 1,1-difenil-2-pikrilhidrazil (DPPH) sebagai sumber radikal bebas. Potensi antihiperglikemik ekstrak kering gambir dianalisis sebagai inhibitor α-glukosidase. Hasil penelitian menunjukkan bahwa beberapa gambir yang ada di pasar Indonesia sangat aktif sebagai antioksidan, yang ditunjukkan dengan nilai IC_{50} dari 4,6 sampai 18,2 μg/mL. Nilai IC_{50} untuk inhibisi α-glukosidase bervariasi antara 40,45 sampai 52,43 μg/mL, dan diklasifasikan sebagai antidiabetes moderat.

Kata kunci: *Uncaria gambier*, antioksidan, antihiperglikemik, ekstrak, α-glukosidase.

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INTRODUCTION

DIABETES mellitus is a serious chronic metabolic disorder and characterized by high blood glucose levels[1]. Five-percent of diabetics have type 1 diabetes, also known as insulin-dependent diabetes mellitus (IDDM) and ninety-five percent of diabetics have type 2 diabetes, and known as non-insulin dependent diabetes mellitus (NIDDM). A third type of diabetes known as gestational diabetes and occur in the last trimester of pregnancy[2].

Worldwide, the number of patients with diabetes are rapidly growing into obesity. At present, it is estimated that 220 milion people worldwide suffering diabetes and this number will increase to 300 million by 2025. According to the WHO, Indonesia is at fourth rank in the number of people with diabetes mellitus, after China, India and USA. Globally, the percentage of type 2 diabetes is more than 90%[3].

Postprandial hyperglycemic plays an important role in the development of type 2 diabetes as an independent risk factor for cardiovascular diseases[4]. One of therapeutic approaches for reducing the postprandial hyperglycemic is to reduce absorption of glucose by the inhibition of carbohydrate hydrolyzing enzymes, for example α-glucosidase, α-amylase, sucrose and maltose. α-Glucosidase is a carbohydrase that catalyzes the liberation of α-glucose from the nonreducing end of the carbohydrate diet. In diabetic patients, inhibition of these enzymes restrain the glucose absorption and reduce the postprandial hyperglycemic[2].

Although acarbose, miglitol, voglibose and nojirimycin are known to be potent inhibitors for α-glucosidase, further screening for α-glucosidase inhibitors from natural sources such as plants and microorganism, will have potency to develop natural pharmaceuticals. A number of antihyperglycemic agents have been found in plants. Research into understanding the scientific basis for plant-based traditional medicine from various culture has increased as scientists search for clues to discover new therapeutic drugs for type 2 diabetes. These plants typically are rich in phenolic compounds, which are known to interact with proteins and inhibit the α-glucosidase and α-amylase enzymatic activity[5,6]. Pine bark extract which is rich in polyphenols such as catechin, quercetin, dihydroquercetin,and phenolic acids, is reportedly effective in supressing postprandial hyperglycemic in diabetics[7].

Gambier is a dried aqueous extract of leaves and branches of gambier plant (*Uncaria gambier* Roxb). Gambier plant is classified into the family of *Rubiaceae*, genus of *Uncaria*[8]. The gambier species is commonly found in Indonesia and used as traditional medicine for burned skin, digestic problem, fever, headache and as antibacterial or antifungi[9-12].

Research on phytochemistry of the *Uncaria* genus has been started in 1900's, which more than 150 chemical compounds were isolated, identified and reported to have pharmacological activities such as antioxidant, antiinflamatory, cytotoxic and immunostimulant[13]. Researchers have published the utilization of poliphenolic compounds of gambier. Catechin was reported to have antioxidant and Gram positive antibacterial activity against *Staphylococcus aureus, Bacillus subtilis* and *Streptococcus mutan*[14-16].

In Indonesia, gambier is commonly chewed by elderly together with betel nuts. Gambier is also utilized as medicine for diarrhea, dysentery, sprue and used also as gargle liquid, antiseptic, cosmetic mixture, adhesive for tanner and dye. Commercial gambier is produced by boiling gambier leaves, compressing and drying. The main phenolic compound of gambier is catechin. In world trade, catechin content is used as a parameter to express the quality of gambier. The minimal catechin content of first, second and third quality grade of gambier are 40%, 30% and 20%, respectively[17]. In international market, Indonesia is the third and the seventh biggest exporter of crude and processed gambier, respectively[18]. Gambier, until now, is exported as crude extract with low selling price. To the contrary, refined gambier catechin extract is traded with high selling price.

The present study investigated the free radical scavenging and antihyperglycemic activities of commercial gambiers sold in local Indonesian markets. The free radical scavenging activity analysis was performed by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) as sources of free radical, that capture hydrogen from gambier dried extract containing catechin as antioxidant. The colour of DPPH turned from purple to yellow, indicating the conversion into 1,1-difenil-2-pikrilhidrazin[19]. The antihyperglycemic activity of gambier dried extracts as α-glucosidase inhibitor was evaluated by using α-glucosidase enzyme and *p*-nitrophenyl α-D-glucopyranoside as a substrate[7]. α-glucosidase will hydrolyse *p*-nitrophenyl α-D-glucopyranoside to become *p*-nitrophenol and glucose. Enzyme activity is measured based on the *p*-nitrophenol yellow colour.

MATERIALS AND METHODS

MATERIALS. Materials used were crude gambiers that were bought from Payakumbuh, Padangpanjang

and Lampung local markets; commercial gambiers from Jakarta that were packed, labelled and sold as SP, Super and Spr brands; ethyl acetate technical and analytical grade chemicals.

Equipment used in this investigation were extraction unit, 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 (Mariner Biospectrometry) using Electrospray Ionization (ESI) System and positive ion mode. DPPH radical scavenging and α-glucosidase inhibitory activities were evaluated by using spectrophotometer Spectronic Hitachi U2000.

METHODS. Isolation of catechin. Catechin was isolated from gambier by extraction. Approximately 500 g of gambier powder, 100 mesh particle size, was put into a 1 kg capacity extractor unit and 1.5 L of technical grade ethyl acetate was added. Solvent rich of catechin was then evaporated using vacuum rotary evaporator to obtain dry extract. The weight and the yield of the dried extract were then calculated. The catechin content of gambier was the yield of extraction process which was formulated as the dry weight of extracted material divided by the weight of crude gambier, multiplied by 100%[20]. The water content of crude gambier and dried gambier extract were also analyzed. Water content analysis was performed by thermogravimetric method based on SNI 01-2891-1992[17].

Catechin content analysis. The dried extract was analyzed by TLC and then compared to catechin standard. The mass or molecular weight (MW) analysis was conducted by LC-MS, whilst the analysis of catechin content was carried out by using spectrophotometric method according to SNI 01-3391-2000[17].

Analysis of free radical scavenging activity. The antioxidant activity was analyzed according to the Yen and Chen's method[19]. Crude gambier and dried extract of gambier were dissolved in methanol to obtain final concentration of 10-200 µg/mL, whilst 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 of DPPH solution in methanol with total volume of 10-25 mL. The blank was 1 mM DPPH in 2.5 mM of methanol. Incubation was carried out at 37°C for 30 minutes and the absorbance was measured spectrophotometrically at 517 nm. The percentage of inhibition was calculated as follows: %inhibition = [(C-S)/C] x 100%, where C is absorbance of the blank and S is the sample absorbance. IC_{50} was calculated as the concentration that caused 50% inhibition of DPPH activity.

Analysis of antihyperglycemic activity of samples. Inhibition analysis on α-glucosidase activity was performed as follows: the α-glucosidase enzyme was dissolved in phosphate-buffer solution (pH 7) containing 200 mg of serum albumin. 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. The sample concentrations for activity evaluation were 3.125, 6.25, 12.5 and 25 µg/mL. The reaction mixture then was incubated at 37°C for 5 min. About 250 µL of the enzyme solution was added into it, and keeps the solution mixture incubated for 15 min. The enzyme reaction was stopped by addition of 1000 µL of 200 mM sodium carbonate solution. The resulted *p*-nitrophenol from the reaction then measured at λ 400 nm. Standard quercetin solutions of same concentrations were also prepared. 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. IC_{50} was calculated as the concentration that caused 50% inhibition of enzyme activity.

RESULTS AND DISCUSSION

The yield of extracted material obtained from crude gambier sample collected from Payakumbuh was the highest (41.8%), commercial gambier Spr Brand was 35.71%, while others were about 20%. According to the catechin content presented by the dried extract, the crude gambier from Payakumbuh was classified into first quality, Spr was second quality, whilst the others were third quality[17]. The low quality most of the crude gambier is possible because of the processing of gambier production were still carried out by traditional extraction process and less hygienic. The catechin content as calculated by yield of catechin extraction from gambier were presented in Figure 1.

The water content of crude gambier and gambier dried extract was also analyzed, to determine the influence of water content on the yield of resulting extract. Based on the water content of crude gambier showed that the smaller of water content, the higher of quality gambier, which means that the higher the catechin content. The water content of crude gambier is presented as Figure 1.

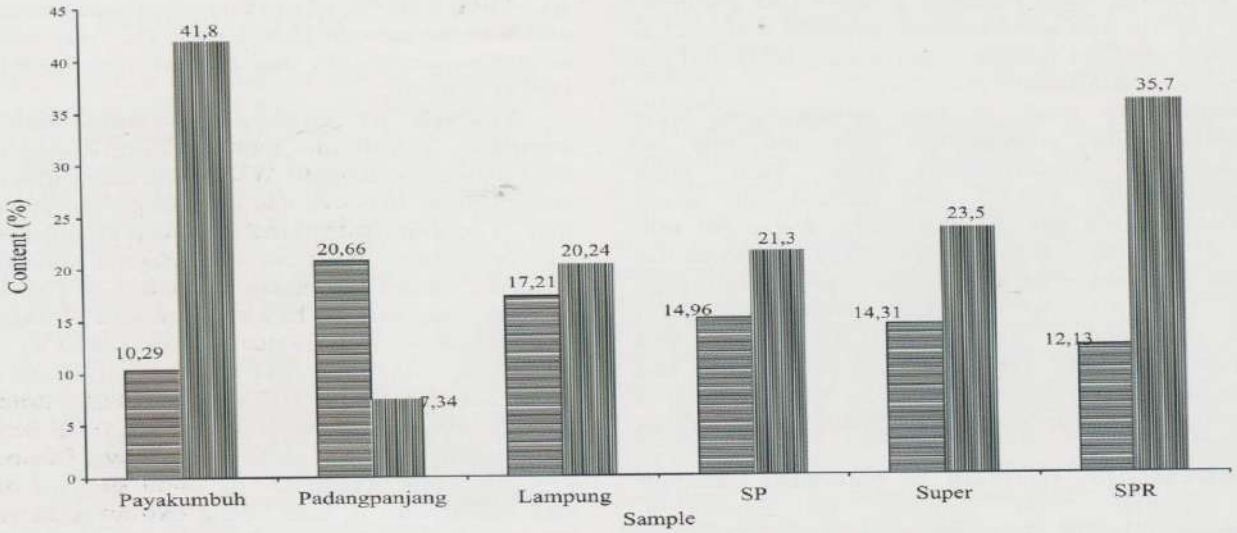

Figure 1. Water content and catechin content of crude gambiers as calculated from the extraction yield. ▤: Water content (%); ▥: Catechin content (%).

Table 1. Results of LC-MS analysis and catechin content of gambier dried extracts.

Gambier samples	LC retention time (minute)	Molecular weight	Catechin content (%)
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

Figure 2. TLC Chromatogram of catechin in gambier dried extract. (eluent 100% EtOAc, detected at 254 nm UV light, Rf value 0.56)

The TLC analysis of catechin showed that dried extract of gambiers contained catechin which was indicated by a spot with Rf value of 0.56 equivalent to that of the standard catechin (Figure 2).

The molecular weight of active compounds in gambier dried extracts were calculated based on LC-MS's data. The chromatogram of gambier dried extracts illustrated 2 peaks with a retention time of the dominant peak was about 1.5 minutes, area under curve (AUC) of 45789.7, and molecular weight of 290.27. The compound was postulated as catechin, since the molecular weight of catechin according to literature was 290.25. The second peak at a retention time of 1.2 minutes and AUC of 3996.4 most likely was impurity. The molecular weight (MW) of compounds in gambier dried extracts were presented as Table 1.

The analysis of catechin content in gambier dried extracts were carried out by spectrophotometric method according to the SNI 01-3391-2000[17], and the results were presented as Table 1. Finally, it could be concluded that the purity of the resultant catechin was relatively high, indicating the successfullness of the extraction process.

The DPPH radical scavenging (antioxidant) activity analysis was carried out with various concentration of gambier dried extracts solution, from

10 to 200 µg/mL, in order to determine the IC_{50} value of the tested solutions. An extract was chategorized as active if its IC_{50} value was less than 100 µg/mL[19]. The IC_{50} value of vitamin C standard at the same assay condition was 10.97 µg/mL. The antioxidant activity analysis showed that the crude gambier have antioxidant activities with IC_{50} values ranged from 10.75 to 40.98 µg/mL. The catechin compound from gambier dried extracts showed higher antioxidant activities with IC_{50} values ranged from 4.55 to18.23 µg/mL. With IC_{50} less than 50 µg/mL, both crude gambier and gambier dried extracts were categorized as active antioxidants. Catechin in three gambier dried extracts (Payakumbuh, Padangpanjang and Lampung) showed higher antioxidant activities about 2.1 up to 2.4 times higher than vitamin C, where IC_{50} of vitamin C was 10.97 µg/mL. The reaction of DPPH free radical scavenging by catechin as antioxidant is presented as Figure 3. Results of DPPH radical scavenging analysis are given in Table 2.

The antihyperglycemic activity of the gambier extracts as α-glucosidase inhibitor were evaluated according to the method previously described. An extract was chategorized as active if its IC_{50} was less than 25 µg/mL[7]. The gambier extracts have moderate α-glucosidase inhibitory activities with IC_{50} values from 40.65 to 52.43 µg/mL. Whilst IC_{50} for α-glucosidase inhibition of standard quercetin was 2.12 µg/mL. The result of the antihyperglycemic activity evaluation showed that gambier dried extract containing catechin has inhibition effect on α-glucosidase enzyme activity, so gambier catechin has potency as an antidiabetic. The result of α-glucosidase inhibition activity analysis are given as Table 2.

Water content has effect to yield of extraction of crude gambier, but doesn't effect to DPPH inhibition and α-glucosidase inhibitory activity of catechin of gambier. The results water content analysis of crude gambier and gambier dried extract presented as Table 2.

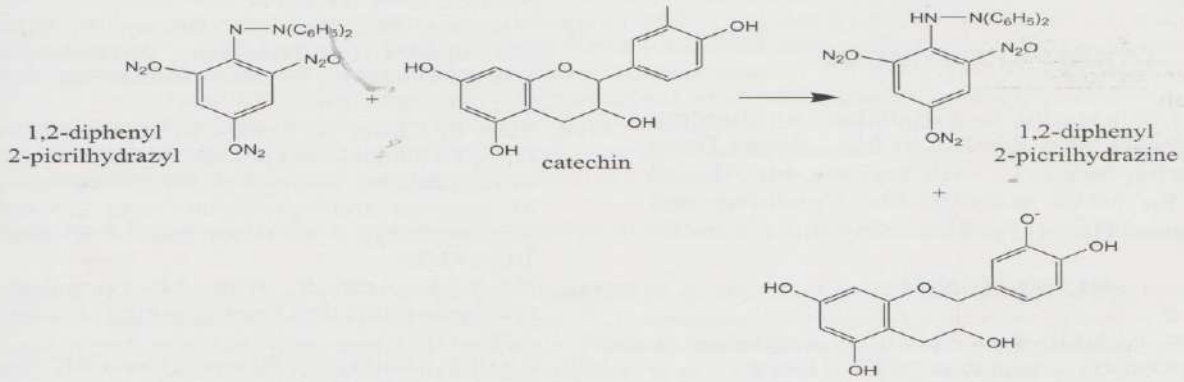

Figure 3. Reaction of catechin with DPPH free radical.

Table 2. The results of DPPH and α-glucosidase inhibition activities and water content of crude gambier and gambier dried extract.

Sample	Water Content (%)	IC_{50} (µg/mL)	
		DPPH	α-Glucosidase
Crude Payakumbuh gambier	10.29	10.75	83.47
Dried extract of Payakumbuh gambier	5.88	4.88	47.57
Crude Padangpanjang gambier	20.66	40.98	89.87
Dried extract of Padangpanjang gambier	3.00	5.10	47.47
Crude Lampung gambier	17.21	15.82	75.31
Dried extract of Lampung gambier	13.15	4.55	48.00
SP brand commercial gambier	14.96	28.46	66.22
Dried extract of SP brand gambier	9.91	18.22	52.43
Super brand commercial gambier	14.31	22.13	70.47
Dried extract of super brand gambier	8.18	14.71	45.77
Spr brand commercial gambier	12.13	30.61	69.12
Dried extract of Spr brand gambier	4.11	19.82	40.65
Ascorbic acid (Vitamin C)	not applicable	10.97	not applicable
Quercetin	not applicable	not applicable	4.08

CONCLUSION

The major bioactive compound of gambier was catechin. According to the catechin content as calculated from the extraction yield of the catechin in various crude and commercial gambier samples in local Indonesian market, most of the gambier samples were third quality with about 20% catechin content. Based on the catechin content of dried gambier extracts of above 90%, it could be concluded that the purity of the resultant catechin was relatively high. The crude and extract of commercial gambier samples have high ability to scavenge reactive free radicals such as DPPH and hence, have high antioxidant activity. The IC_{50} of catechin that were extracted from various gambier samples in local Indonesian market, ranged from 4.55 to 18.2 μg/mL, therefore they were categorized into very active antioxidant. The antioxidant activity of three extracts from Payakumbuh, Padangpanjang and Lampung gambier samples were higher, about 2.1 to 2.4 times of vitamin C.

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