



In Vitro Alpha Glucosidase Activity of *Uncaria gambir* Roxb. and *Syzygium polyanthum* (Wight) Walp. from West Sumatra, Indonesia

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Abstract

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BACKGROUND: Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia that occurs due to abnormal insulin secretion, insulin action, or both. Alpha-glucosidase enzyme inhibitors are drugs that work by slowing the absorption of carbohydrates in the intestine so that it has an impact on decreasing post-prandial glucose levels.

AIM: The aim of the study was to assess the alpha-glucosidase inhibitory activities of methanolic extracts from different parts of *Uncaria gambir* Roxb. and *Syzygium polyanthum* (Wight) Walp. grown in Padang, West Sumatera, Indonesia often used as herbal medicine.

MATERIALS AND METHODS: Methanolic extracts from different parts, fractions, and different drying methods of *U. gambir* Roxb. and *S. polyanthum* (Wight) Walp. grown in Padang, West Sumatera, Indonesia, were prepared for alpha-glucosidase inhibitory activities.

RESULT: The proanthocyanidin fraction of *U. gambir* Roxb. (purity of $\geq 75\%$) showed a potent α -glucosidase inhibitory activities as evidenced by low IC_{50} at $28,993 \pm 1,377 \mu\text{g/ml}$, while (+)-catechin at $30,855 \pm 0,791 \mu\text{g/ml}$ (IC_{50} acarbose: $111,286 \pm 2,386 \mu\text{g/ml}$). The Butanol fraction of *S. polyanthum* (Wight) Walp. showed potent α -glucosidase inhibitory activities as evidenced by low IC_{50} at $28,469 \pm 1,929 \mu\text{g/ml}$.

CONCLUSION: Butanol fraction from *U. gambir* Roxb.'s oven-dried leaves and *S. polyanthum* (Wight) Walp. leaves contained proanthocyanidin class compounds. The proanthocyanidin fraction with a purity of $> 75\%$ had a stronger alpha-glucosidase inhibitory activity than the acarbose as a positive control.

Introduction

Large numbers of medicinal herbs were identified in Indonesia. These herbs, leaves, stems, or roots are used as a source of medicinal products. The benefit of these medicinal herbs is not only related to the secondary metabolites produced by these plants as a defense mechanism against injury and infection but also play a vital role in the medicinal field, because of its healing capacity. The content of secondary metabolites active in herbs can vary among others due to plant species, the country of origin, its growth phase, and seasonal changes. These secondary metabolites include among others: Phenolic, flavonoids, and their derivatives (tannins, proanthocyanidins, and vitamins) [1].

Phenolic contents are known to be the most important class of phytochemicals in the plant. They can be classified into two main groups, polyphenols and flavonoids. Phenolics are usually associated with the inhibition of oxidative stress-related diseases such as diabetes and cancer in humans. Flavonoids are polyphenolic contents, commonly found in plants. Besides being a potential antioxidant compound, flavonoid also exhibits potential pharmacological activities [2].

Proanthocyanidins or condensed tannins are flavonoid oligomers that are widely distributed in plants. They have beneficial effects on human health. Flavan-3-ols such as catechin are a component of proanthocyanidins and serve as flavor and astringency in tea also exhibit potential pharmacological activities such as anticarcinogenic.

Drying is basically defined as a process of water removed and decreasing of herbs moisture content, aimed at preventing microbial and enzymatic activity, consequently preserving the product for extend shelf life. Understanding at what level different drying techniques may influence the content of compounds with a proven effect on human organism may lead to postharvest management of these compounds profile in the future. There are some published data reports on various drying processes of *U. gambir* (*Uncaria gambir* Roxb.) leaf which have an impact on compound variations and their bioactivity [3], [4]. Thus, the search for potential keeping the phytochemical in these herbs has gained increasing interest among researchers. In literature, there is only a small amount of information about the changes of phenolic compounds and antioxidant potential in herbs in the context of drying together with the variability of their composition and biological activity.

Diabetes is a common disease in developing countries and developed countries. DM (diabetes mellitus) is a metabolic disorder with multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The global prevalence was estimated approximately 382 million people in 2013 suffer from DM and it is projected that it will increase to 592 million in 2035 if no action is taken [5].

Some management strategy diabetes is by giving insulin, correct disorders of insulin in the pancreas (sulfonylureas, glinides, incretin mimetic, and inhibitors of DPP4 [dipeptidyl peptidase-4]), repair impaired insulin on the insulin receptor (Biguanides and Thiazolidinediones), and slowing digestion and absorption of carbohydrates in the intestines to maintain the level post-prandial glucose (inhibitor of alpha-glucosidase and alpha-amylase). Alpha-glucosidase is an enzyme found in the brush border of the small intestine (intraluminal). This enzyme works by breaking the disaccharide into monosaccharides. Monosaccharides will be absorbed into the blood vessels, then blood glucose levels rise. Acarbose is inhibitors of the intestinal brush border α glucosidases. It is an oligosaccharide analog that binds more avidly than natural carbohydrate to the intestinal disaccharides such as α glucosidase. Hence, the absorption of most carbohydrates is delayed. This competitive inhibition of α glucosidase limits the post-prandial rise of glucose. Unfortunately, current antidiabetic therapies are based on synthetic drugs that very often have side effects such as hypoglycemia, weight gain, gastrointestinal disturbances, nausea, diarrhea, liver, and heart failure [6]. Therefore, antidiabetic agents which can provide a minimal effect on the side effects are a challenge in this century. Some herbs used by a traditional society as the lowering of blood sugar agent is thought to have minimal side effects.

Fresh herbs (*U. gambir* and *S. polyanthum*¹) are an excellent source of phenolic compounds which have been reported to show good α glucosidase inhibitory activity [7], [8]. Therefore, our present study reveals the qualitative measurement of potential natural constituents and quantification total phenolics, flavonoids, proanthocyanidins, and α glucosidase inhibition activity of different parts *U. gambir* and *S. polyanthum* in Padang, West Sumatra area [9], [10], [11]. Furthermore, in this work, we evaluated the results of three drying methods of *U. gambir* leaves and its derived fractions and compound in relation to active groups (phenolics, flavonoids, and proanthocyanidins) and biological activity (α glucosidase inhibitory activity) [12].

Materials and Methods

Identification and plant material

Five kilograms fresh and healthy leaves, stems, and roots of *U. gambir* and *S. polyanthum* were collected from KTOF (Kebun Tumbuhan Obat Farmasi), Sumatran Biota Laboratory, Andalas University. The sample specimen was identified based on the taxonomical characteristics and deposited in the herbarium of Andalas University. The leaf, stem, and root were washed thoroughly in distilled water and the surface water was removed by air under shade. All of the samples were dried according to the method described by Roshanak, Sudibyo and Joseph, and Chua *et al.* with a slight modification, on a naturally dried by air under shade (20°C, 3 days), except for gambir leaves [13], [14], [15]. The leaves were dried using one of the following drying methods; oven (50°C, 24 h), steam blanching (100°C, 10 min) by a household steam cooker followed by naturally dried by air under shade (25°C, 3 days), and naturally dried by air under shade (25°C, 3 days). After that, all of the samples were milled into powder by a grinder. The resulting powders were immediately packaged and kept in the dark place before extraction and analyses.

Chemicals

α glucosidase from *Saccharomyces cerevisiae* (EC 3.2.1.20), substrate p -nitrophenyl- α -D-glucopyranoside (pNPG) were purchased from Sigma Aldrich, Singapore. (+)-Catechin was from *U. gambir* Roxb. The purity of the isolated (+)-catechin was approximately 99.9% and was purchased from PT. Andalas Sitawa Fitolab. All other chemicals were analytical grade and purchased from Sigma Aldrich, Singapore.

Preparation of methanol extract and proanthocyanidin fraction

The powdered leaves, stems, and root of *U. gambir* and *S. polyanthum* were macerated in methanol 70%. Maceration took 9 days at room temperature and done by changing the solvent in every 3 days. The methanol extract was subsequently filtered through Whatman No. 1 filter paper and transferred into the sample holder of the rotary flash evaporator for the evaporation of the solvent to obtained extraction yields of *U. gambir*'s naturally dried leaf (24,99%), *U. gambir*'s oven-dried leaf (18,65%), *U. gambir*'s steam dried leaf (46.11%), *U. gambir*'s stems (8,95 %), *U. gambir*'s roots (15,28 %), *S. polyanthum*'s leaf (19,39 %), *S. polyanthum*'s stems (22,78 %), and *S. polyanthum*'s roots (7,63 %).

Five hundred grams of methanol crude extracts of *U. gambir*'s oven dried leaf, *U. gambir*'s stem dried leaf, *S. polyanthum*'s leaf, and *S. polyanthum*'s stems were then fractionated successively with hexane, ethyl acetate, and

1 *S. polyanthum* : *Syzygium polyanthum* (Wight) Walp

butanol to obtained fractionated yields of hexane fraction of *U. gambir's* oven dried leaf (0%), ethyl acetate fraction of *U. gambir's* oven dried leaf (40,6%), butanol fraction of *U. gambir's* oven dried leaf (10%), hexane fraction of *U. gambir's* stems (5,10%), ethyl acetate fraction of *U. gambir's* stems (20,4%), butanol fraction of *U. gambir's* stems (3,06%), hexane fraction of *S. polyanthum's* leaf (0%), ethyl acetate fraction of *S. polyanthum's* leaf (50%), butanol fraction of *S. polyanthum's* leaf (13%), S. hexane fraction of polyanthum's stems (3,10%), ethyl acetate fraction of *S. polyanthum's* stems (24,76%), and butanol fraction of *S. polyanthum's* stems (9,30%). Ten grams of obtained butanol fraction of *U. gambir's* oven-dried leaf were directly subjected to a Diaion HP-20 (Sigma Aldrich, Singapore) column (9 cm i.d. × 60 cm) with H₂O containing increasing amounts of MeOH in stepwise gradient mode and then fractionated into 10 subfractions SA01–SA10, respectively. Of the obtained subfractions, SA03 was a proanthocyanidin fraction, a brown powder 31,2 mg (0.32%). All of the extracts were preserved in a brown bottle until further use.

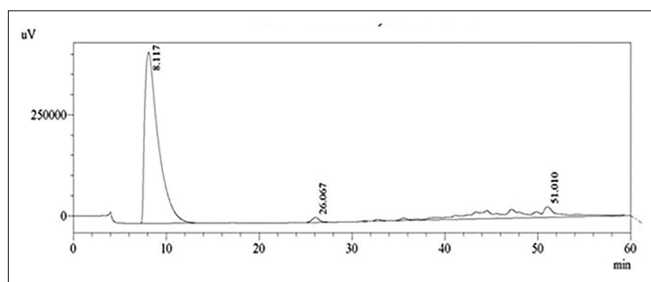


Figure 1: The purity of proanthocyanidin fraction was determined using HPLC analysis. The amount was measured by HPLC technique. The peak proanthocyanidin fraction was observed at a retention time of 8,117 min

HPLC (High-performance Liquid Chromatography) analysis

The HPLC system (Shimadzu, Japan) consisted of a binary gradient pump (LC- 10AD). The quantification of proanthocyanidin fraction was set at 254 nm. The chromatographic separation was performed at ambient temperature (25–28 C) using Hypersil BSD C18 column (4,6 × 100 mm, 3 μm size) (Thermo Scientific, USA) with a C18 guard column. The mobile phase consisted of water (A) and methanol (B) were delivered at flow rate of 1000 ml/min following programmed gradient elution: 100% (A) isocratic for 5 min, 90% (A) for 5 min, 80% (A) for 5 min, 70% (A) for 5 min, 60% (A) for 5 min, 50% (A) for 5 min, 40% (A) for 5 min, 30% (A) for 5 min, 15% (A) for 5 min, 5 % (A) for 5 min, and 0% (A) isocratic for 10 min post-run for reconditioning. Sample volume injection was adjusted to 10 μL. Total running time was 60 min. All solution of the mobile phase were freshly prepared, filtered through 0.45 μm Nylon filter under vacuum and degassed by sonication for 20 min before use. The result was analyzed using ChemStation software. The percentage of proanthocyanidin fraction was ≥ 75% calculated based on the peak area (Figure 1).

Qualitative phytochemical analysis

The viscous extracts (50 mg) were diluted using methanol. Identification of the presence of phenolic, alkaloid, saponin, steroid, terpenoid, and flavonoid conducted in accordance with procedures of Harborne [16].

Evaluation of bioactive constituents

Total phenolic content

A Folin-Ciocalteu method based on Slinkard and Singleton report was applied to determine the total amount of phenolic compounds in methanol extract from different parts of *U. gambir* and *S. polyanthum* [17]. The reduction of Folin-Ciocalteu reagent by phenolic ion will change its solution color into blue. The reduction of complex will increase when the extract contains more phenolic compounds. Thus, the color will be darker and the absorbance will be higher. Samples 50 μl of each plant part was added to 50 μl aquadest, 50 μl of 7,5% Folin-Ciocalteu in aquadest, incubated at 37°C for 8 min. Finally, 50 μl of 1% sodium hydroxide was added and incubated at 37°C for 60 min. Absorbance values of the solutions were measured at 750 nm. Total phenolic contents were determined as a gallic acid equivalent (GAE) based on Folin-Ciocalteu calibration curve using gallic acid as the standard and expressed as mg gallic acid per 100 g of dry mass (% b/b).

Flavonoid content

The total soluble flavonoid contents of methanol extract of different parts of *U. gambir* and *S. polyanthum* were estimated by the spectrophotometric method [18]. The aluminum chloride forms acid-stable complexes with the hydroxyl group of the flavonoids and flavonols. 0.5 ml of stock solution of the extract, 1.5 ml methanol, 0.1 ml of 10% aluminum chloride, and 0.1 ml of 1M sodium acetate was added to reaction tubes and volume was made up to 5 ml with distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm. Total flavonoid content was calculated by extrapolating the absorbance of the reaction mixture on a standard curve of rutin. The total flavonoid content was expressed as equivalent to rutin in % b/b of the extracts.

Proanthocyanidin content

Proanthocyanidins in the sample solutions of methanol extract from different parts of *U. gambir* and *S. polyanthum* were determined by the vanillin-HCl assay described by Sun *et al.* [19]. The vanillin reaction involves the reaction of an aromatic aldehyde, vanillin, with the meta substituted ring of flavanols to yield a red adduct [20]. To 100 μl of test solution or methanol (control) in a test tube, 2.5 ml of 8% hydrogen chloride

in glacial acetic acid and 2.4 ml of 1% vanillin in glacial acetic acid solution was added. The reaction mixture was incubated for 5 min at room temperature and the absorbance at 510 nm was measured. The data for proanthocyanidin contents were expressed as mg of (+)-catechin equivalent weight (CAE)/ 100 g of dry mass (% b/b).

***α*lpha-glucosidase inhibitory activity**

The *α*lpha-glucosidase inhibitory activity of methanol extract from different parts of *U. gambir* and *S. polyanthum* was determined according to the method described by Mohamed *et al.* with a slight modification [21]. A mixture of 50 μ l of the sample solution and 100 μ l of 0.1 M phosphate buffer containing *α*lpha-glucosidase solution (1 U/ml) was incubated in 96 well plates at 25°C for 10 min. After preincubation, 50 μ l of 5 mM pNPG solution in 0.1 M phosphate buffer was added to each well at timed intervals. The reaction mixtures were incubated at 25°C for 5 min. Absorbance was recorded at 405 nm by a microplate reader (Bio-Rad). Acarbose was used as a positive control. The *α*lpha-glucosidase inhibitory activity was expressed as inhibition percent and was calculated as follows:

Inhibition (%) = $(A_{ref} - A_{sam})/A_{ref} \times 100$; where A_{ref} is the absorbance of the reference; A_{sam} is the absorbance of the test samples.

Statistical analysis

All data were conducted in triplicate. The reported value for each sample was calculated as the mean \pm SE of three measurements.

Results and Discussion

Extraction yields and phytochemical analysis

Percentage yield (% w/w) was determined by comparing the weight of the dried leaves with the weight of the crude extract produced. The highest extraction yield of methanol extract was found on *U. gambir*'s steam bleaching leaf extracts (46,11%) followed by *U. gambir*'s naturally (24.99%) and oven-dried leaf extracts (18,65%). Thermal treatment is considered as an efficient method to inactivate enzymes. Conventionally, steam blanching is normally applied for enzyme inactivation [22]. Our result proved that steam bleaching resulted in increases in the yields. One possible explanation is that a short blanching could be beneficial for the texture of the leaf for the extraction process.

S. polyanthum's stem extracts and *U. gambir*'s leaf extracts showed higher yields than other extracts

compared on the same species. These differences could be attributed to the high levels of the total component in *S. polyanthum*'s stem extracts and *U. gambir*'s leaf extracts. Moreover, *S. polyanthum*'s stem extracts and *U. gambir*'s leaf extracts are seemed to be rather narrow compared with other parts, morphologically, thus facilitates its extraction. These reasons also apply to *U. gambir*'s leaf extracts.

Phytochemical screening is the major tool to bring about the wonderful potential of plants. Plant metabolites were responsible for potential activities. Phytochemical screening in this study was aimed at detecting the presence of phenolic, alkaloid, saponins, steroid, terpenoid, and flavonoid. General reactions revealed the presence or absence of these compounds in the tested extracts. The resulting qualitative analysis showed that all parts of *U. gambir* contained phenolic, alkaloids, and flavonoids. Similar observations have been made by Thorpe and Whiteley and Amir *et al.*, while saponin, steroid, and terpenoid were not detected in the overall part of *U. gambir* (Table 1) [23], [24]. Saponin and steroid only found on *U. gambir*'s oven-dried and steam bleaching leaves, yet terpenoid found on all of *U. gambir* part extracts, except leaves dried steam process. Among the extracts, *U. gambir*'s oven-dried leaf provided a strong positive for the natural constituents such as phenolic, alkaloid, saponin, steroid, terpenoid, and flavonoid.

All parts of *S. polyanthum* contained phenolic and flavonoid, while terpenoid and steroid were not detected in overall parts of *S. polyanthum*. Saponin only detected on leaves and stem of *S. polyanthum* and not found on the root. This result is contrary to the previous literature. Kusuma *et al.* found that *S. polyanthum*'s leaf grown in Samarinda and Balikpapan, Indonesia did not contain saponin, but flavonoid, alkaloid, tannin, glycoside, terpene, and steroid. *S. polyanthum*' leaf grown in Medan, Indonesia, contained flavonoid, alkaloid, tannin, and glycoside [25], [26].

Total phenolic, flavonoid, and proanthocyanidin contents

There are more than 8000 phenolic compounds, which are distributed into classes based on the number of phenolic rings in the structure and the constituents that bind to the rings. Phenolic compounds are further categorized as flavonoids and non-flavonoids. The subgroups within the flavonoid category include flavonols,

Table 1: Phytochemical screening contents

Samples	Phenolic	Alkaloid	Saponin	Steroid	Terpenoid	Flavonoid
<i>U. gambir</i> 's naturally dried leaf	+	+	-	-	+	+
<i>U. gambir</i> 's oven-dried leaf	+	+	+	+	+	+
<i>U. gambir</i> 's steam dried leaf	+	+	+	+	-	+
<i>U. gambir</i> 's stems	+	+	-	-	+	+
<i>U. gambir</i> 's root	+	+	-	-	+	+
<i>S. polyanthum</i> 's leaf	+	-	+	-	+	+
<i>S. polyanthum</i> 's stem	+	-	+	-	+	+
<i>S. polyanthum</i> 's root	+	-	-	-	+	+

+: Present - Absent.

flavones, isoflavones, flavanones, anthocyanidins, and flavanols. The flavanols subclass includes catechins and proanthocyanidins [27]. The total phenolic, flavonoid, and proanthocyanidin contents of 8 methanolic extracts from 2 Indonesian medicinal plant species were systematically assessed. Phenolic compounds were found to be the major components of all extracts.

The methods of drying affected the level of phenolics, flavonoids, and proanthocyanidin compounds in analyzed herbs. *U. gambir's* steam blanching leaf extract ($42,536 \pm 1,047\%$ GAE) provided the highest total phenolic content among other parts, followed by *U. gambir's* naturally dried leaf and *U. gambir's* stems extracts which were $29,659 \pm 2,521\%$ GAE and $27,735 \pm 1,455\%$ GAE, respectively. Among three different drying processes, oven-dried process had the lowest phenolic compounds ($17,580 \pm 0,529\%$ GAE). Proanthocyanidin and flavonoid compounds had a different pattern with phenolic compounds. Proanthocyanidin content of *U. gambir's* steam blanching ($37,927 \pm 1,267\%$ CAE), naturally ($14,293 \pm 0,671\%$ CAE), oven-dried leaf ($8,422 \pm 0,466\%$ CAE) and flavonoid content of naturally dried leaf ($32,472 \pm 1,095\%$ RAE), and steam blanching leaf ($29,396 \pm 1,742\%$ CAE).

The effect of steam blanching process as a pre-treatment step on extraction method was tested in this attempt. Similar effects of steaming were reported from Gliszczynska-Swiglo *et al.* where found a 52% increase in the total phenolic content in steamed (10 min) broccoli, explaining this by enhanced extractability due to disruption of the polyphenoloxidase, polyphenol-protein complexes [28]. Moreover, steam blanching process has a positive effect on the extractability of flavonoids since in respective samples, flavonoid yields are higher compared to other samples.

In the case of *U. gambir's* oven-dried leaf, where the lowest content of phenolics, flavonoids, and proanthocyanidins compounds existed, it has been already published that some phenolic compounds in plants may be a part of bigger molecules which do not react with Folin-Ciocalteu reagent [29]. It can be suggested that on oven process, these binds with bigger molecules may be existed and it caused the lowest concentration of phenolic compounds. Other, possibility is the bioactive compounds of *U. gambir's* leaf may be destroyed on oven processed at a high temperature.

According to Rahmawati and Fernando and Jaya *et al.*, *U. gambir's* leaf grown in Kampar, Riau, Indonesia which was dried at 60°C oven had phenolic contents of 15.7% GAE, on the other hand, 43,13% GAE was in phenolic water extracts of gambir product [29], [30], [31]. The amount of catechin, a class of flavonoids present in methanol leaf extract of *U. gambir* contained more catechin compounds 8,635% CAE than stem parts 5,120% CAE [32]. *U. gambir's* leaf extracts grown in Indonesia extracted by steaming (10 min) had the more percentage yields and catechin contents than without pre-treatment processes [14]. Our resulting data were almost the same as previous researches.

Total phenolic and proanthocyanidin contents assay in the present study revealed that *S. polyanthum's* stem extract had the highest total phenolic and proanthocyanidin contents among *S. polyanthum* extracts which were $23,846 \pm 0,613\%$ GAE and $23,336 \pm 1,192$ followed by root parts $21,336 \pm 0,810\%$ GAE and $14,156 \pm 1,284$, the last was leaves extract $15,763 \pm 0,588\%$ GAE and $6,648 \pm 0,715$, respectively. Nevertheless, flavonoid content was not followed phenol and proanthocyanidins's pattern. This study showed that total flavonoid content in the *S. polyanthum* extract was ranked as follows leaves ($38,633 \pm 4,926\%$ RAE) > stems ($18,000 \pm 0,495\%$ RAE) > roots ($12,088 \pm 1,697\%$ RAE).

According to Wong *et al.*, water extract of *S. polyanthum's* leaf collected in Singapore contained 1,1 % GAE dry weight total phenolic content [33]. In contrast, methanol extracts of *S. polyanthum's* leaf in the present study contained higher total phenolic content. The difference could be attributed to the high levels of total phenolic content in the *S. polyanthum's* leaf grown in Padang, West Sumatra, or due to the different method of extraction employed.

The calculation of total phenolic, flavonoid, and proanthocyanidin content of *U. gambir* and *S. polyanthum* extracts was presented in Table 2.

Table 2: Total phenolic, flavonoid, and proanthocyanidin contents

Sample	Total phenolic contents (% GAE ²)	Flavonoid content (% RAE ³)	Proanthocyanidin content (%CAE ⁴)
<i>U. gambir's</i> naturally dried leaf	$29,659 \pm 2,521$	$32,472 \pm 1,095$	$14,293 \pm 0,671$
<i>U. gambir's</i> oven dried leaves	$17,580 \pm 0,529$	$27,352 \pm 1,902$	$8,422 \pm 0,466$
<i>U. gambir's</i> steam dried leaf	$42,536 \pm 1,047$	$29,396 \pm 1,742$	$37,927 \pm 1,267$
<i>U. gambir's</i> stems	$27,735 \pm 1,455$	$67,613 \pm 0,826$	$16,699 \pm 0,544$
<i>U. gambir's</i> roots	$18,088 \pm 0,619$	$32,456 \pm 1,286$	$12,226 \pm 0,371$
<i>S. polyanthum's</i> leaf	$15,763 \pm 0,588$	$38,633 \pm 4,926$	$6,648 \pm 0,715$
<i>S. polyanthum's</i> stem	$23,846 \pm 0,613$	$18,000 \pm 0,495$	$23,336 \pm 1,192$
<i>S. polyanthum's</i> root	$21,336 \pm 0,810$	$12,088 \pm 1,697$	$14,156 \pm 1,284$
Proanthocyanidin fraction	not determined	not determined	$79,946 \pm 1,100$

Means \pm SEM

Inhibition of α -glucosidase

α -glucosidase is an enzyme produced by the villi lining the small intestine of mammals and is responsible for the hydrolysis of disaccharides to monosaccharide that can be absorbed and consequently elevate blood glucose levels. Inhibition of intestinal α -glucosidase has been used successfully as a therapeutic target for modulation of post-prandial hyperglycemia, which is the earliest metabolic abnormality to occur in type 2 DM [34], [35]. The α -glucosidase inhibiting activity of the eight plant extracts was tested following the method described by Mohamed *et al* [21]. This microplate assay offers great convenience, speed, and reproducibility. The α -glucosidase inhibitory activity was determined by measuring a yellow color due to the release of p-nitrophenol from p-nitrophenyl- α -D-glucopyranoside.²

2 % GAE : percent of gallic acid equivalent

3 % RAE : percent of rutin equivalent

4 % CAE : percent of (+)-catechin equivalent

The α -glucosidase 50% inhibitory concentrations (IC_{50}) for the methanol extract of different parts and plants, as well as the positive control, acarbose, was depicted in Table 3. It was evident from the results that *U. gambir*'s oven-dried leaf extracts inhibited α -glucosidase the most with a IC_{50} value of $36,865 \pm 1,187 \mu\text{g/ml}$. IC_{50} value of $111,286 \pm 2,386 \mu\text{g/ml}$ was obtained for the positive control, acarbose. Interestingly, all extracts tested were more potent inhibitors of α -glucosidase than acarbose.

Table 3: Inhibitory effect (IC_{50}) of samples and positive control acarbose for alpha-glucosidase

Sample	IC_{50} ($\mu\text{g/ml}$) ^a
<i>U. gambir</i>	
Leaf	
Naturally dried	40,194 \pm 2,437
Oven dried	36,865 \pm 1,187
Ethyl acetate fraction	29,474 \pm 0,485
Butanol fraction	20,471 \pm 0,705
Steam dried	40,278 \pm 1,500
Stem	
Hexane fraction	201,688 \pm 2,194
Ethyl acetate fraction	37,479 \pm 0,467
Butanol fraction	23,331 \pm 1,637
Root	
44,683 \pm 2,637	
<i>S. polyanthum</i>	
Leaf	
Ethyl acetate fraction	53,431 \pm 1,498
Butanol fraction	35,861 \pm 2,384
28,469 \pm 1,929	
Stem	
56,593 \pm 2,475	
Hexane fraction	233,861 \pm 2,888
Ethyl acetate fraction	29,124 \pm 0,450
Butanol fraction	30,465 \pm 0,502
Root	
58,222 \pm 3,751	
(+)-Catechin	30,855 \pm 0,791
Proanthocyanidin fraction	28,993 \pm 1,377
Acarbose	111,286 \pm 2,386

^aAverage of three determinations, mean \pm SEM (n=3)

Previous reports have shown that bioactive compounds such as flavonoids and phenolics, from a wide range of food or medical plant sources, could be effective α -glucosidase inhibitors [36]. Our study demonstrated that the IC_{50} value of the α -glucosidase activity of *U. gambir* extracts was not correlate with the level of phenolic, flavonoid, and proanthocyanidin contents. Therefore, it is rationally presumed that extracts may have other different active components.

Based on Table 3, *U. gambir*'s oven-dried leaf extracts exhibited the highest α -glucosidase inhibitory activity among the extracts, meanwhile its phenolic, flavonoid, and proanthocyanidin content were the lowest among other *U. gambir* extracts. Benalla et al. [9] suggested that many kinds of natural products show strong activity against α -glucosidase such as alkaloids, saponin, steroid, polyphenol, terpenoid, acids (chlorogenic acid, betulinic acid, syringic acid, vanillic acid, bartogenic acid, oleanolic acid, dehydrotrametenolic acid, corosolic acid, ellagic acid, ursolic acid, and gallic acid), phytosterol, myoinositol, flavonoids, flavonolignans, anthraquinones, anthrones, xanthenes, feruloylglucosides, flavanone glucosides, acetophenone glucosides, glucopyranoside derivatives, genine derivatives, flavonol, anthocyanin, and others [37], [38], [39], [40], [41], [42].

It has been mentioned that *U. gambir*'s oven-dried leaf extracts react positively in all phytochemical screening tests. The low relationship confirms that

phenolic, flavonoid, and proanthocyanidin compounds were not the only contributor to the α -glucosidase activities of the extracts. The type and quantity of phenolic compound and the presence of non-phenolic antioxidant contents may contribute to the antioxidant activity of the extracts. Moreover, it could be due to synergistic interaction of phenolics, flavonoid, proanthocyanidin, and also interaction with non-phenolic compounds which may be an important factor in contributing to the total α -glucosidase activity [43].

We demonstrated that all of the *U. gambir* extracts had higher activity than acarbose. Our findings were in line with previous data performed by Kim, who conducted α -glucosidase inhibitory activity assay of eight compounds of aqueous methanolic extract of manufactured gambir product from *U. gambir* collected in Hunan province, China. Kim proved that all of the compounds as a result of isolation along with the Diaion HP-20 column chromatography fraction ($30.3 \pm 2.7 \mu\text{g/ml}$) showed a potent inhibitory activity α -glucosidase (IC_{50} acarbose $312.6 \pm 3.8 \mu\text{M} = 201,42 \mu\text{g/ml}$) [44].

Our study suggested that the IC_{50} value of *S. polyanthum* extracts was not correlate with the level of phenolic, flavonoid, and proanthocyanidin contents. Our data supported the previously published reports. Twenty of the *Syzygium* sp. were tested for its antidiabetic property through α -glucosidase inhibitory assay. The results demonstrated that IC_{50} value of 20 *Syzygium* sp. ranged from 8.71 (*S. cumini*) – 72.63 $\mu\text{g/ml}$ (*S. samarangense*), while quercetin as a standard was IC_{50} 22,39 $\mu\text{g/ml}$ (45). IC_{50} of *S. polyanthum* (19,06 $\mu\text{g/ml}$) collected in Bogor, Indonesia, showed strong activity against α -glucosidase enzyme better than acarbose (36,98 $\mu\text{g/ml}$) (8). Polyphenols were associated with the activity of α -glucosidase inhibitors. The presence of other phytochemical compounds and synergistic action may play a role in α -glucosidase activity of *U. gambir* extracts [46].

U. gambir's oven-dried leaf, *U. gambir*'s stems, *S. polyanthum*'s leaf, and *S. polyanthum*'s stem were fractionated and tested for alpha-glucosidase activity. The results showed that the butanol fraction of *U. gambir*'s oven-dried leaf had the most powerful alpha-glucosidase activity (IC_{50} 20,471 $\mu\text{g/ml}$). It is well-known that the butanol fraction contains more polar compounds than the ethyl acetate fraction [9]. Hence, it can be concluded that the polar component plays a major role in the activity of alpha-glucosidase inhibition.

To find out the polar compound that plays a role in alpha-glucosidase activity, butanol fraction of *U. gambir*'s oven-dried leaf was then prepared by Diaion HP-20 to give a proanthocyanidin fraction (purity of > 75%). The alpha-glucosidase activity of the proanthocyanidin fraction showed an IC_{50} of 28,993 $\mu\text{g/ml}$ and as a comparison, the compound (+)-catechin has an IC_{50} of 30.85 $\mu\text{g/ml}$. The IC_{50}

value of the proanthocyanidin fraction is stronger compared to (+)-catechins because the general structure of the proanthocyanidin group compounds has more OH-groups than (+)-catechins thus making it more polar. This research is in line with Kim who stated that IC_{50} value of procyanidin B3, a group of proanthocyanidin is 17.3 μ M and (+)-catechin was 53.8 μ M, while acarbose was 312.6 μ M. Kim showed that an IC_{50} value of proanthocyanidin has a strength almost 3x than (+)-catechin [44]. This is different from our study because the components we tested were proanthocyanidin fraction, so there may a role for other components as inhibitors of alpha-glucosidase enzyme.

Conclusion

In the present study, different parts of *U. gambir* extracts, *S. polyanthum* extracts, and *U. gambir*'s derived fraction and compound possessed inhibitory activity against α -glucosidase *in vitro*. Moreover, phenolics, flavonoids, and proanthocyanidins were found to be partly responsible for bioactivities of these extracts. These results indicated that both of *U. gambir* and *S. polyanthum* extracts could be utilized as a renewable bioresource to develop potential antidiabetic agents. Furthermore, methanol extraction could be an effective step for obtaining natural antioxidants from these samples, although further optimization of extraction is required.

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