

Alpha-glucosidase and DPP-IV Inhibitory Activities of Ethanol Extract from *Caesalpinia sappan*, *Andrographis paniculata*, and *Syzygium cumini*

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ABSTRACT

Background: Diabetes is one of the fastest-growing global health problems of the 21st century. Antidiabetic medicine has been widely marketed with various mechanisms of action. However, there are side effects from these drugs. Therefore, most diabetic patients consume herbal as complementary. Plants that have been shown to have potential as an antidiabetic are *Caesalpinia Sappan*, *Andrographis Paniculata* and *Syzygium Cumini*. **Objective:** This study aims to examine the in vitro antidiabetic activity of single and combined ethanol extract of those three plants by inhibiting alpha-glucosidase and DPP-IV (Dipeptidyl peptidase IV) enzymes. **Materials and Methods:** The alpha-glucosidase inhibitory activity was determined using the paranitrofenil alfa-D-glukopiranosida (pNPG) reaction at a wavelength of 405 nm. Acarbose was used as the positive control. The DPP-IV inhibitory activity using H-Gly-Pro-AMC substrate and detected by fluorescence at $\lambda_{ex} = 365$ nm and $\lambda_{em} = 415-445$ nm. Sitagliptin was used as the positive control. LC-MS analysis was performed to identify the compounds contained in the combined extract. **Results:** *Caesalpinia sappan* showed better activity to inhibit alpha-glucosidase enzyme than acarbose at IC_{50} of 9,29 μ g/mL. The combined extract obtained higher inhibition as DPP-IV inhibitor than single extract at 63, 69%. The highest compound in the combined extract were 5,7-Dihydroxy-3-(4'-hydroxybenzyl) chromone, Protosappanin E-1, Saurufuran B and candidate mass $C_{36}H_{38}N_4O_5$. **Conclusion:** These results indicate that single extract or combined extract potential as antidiabetic.

Key words: Alpha-glucosidase inhibitor, *Andrographis paniculata*, *Caesalpinia sappan*, DPP-IV Inhibitor, *Syzygium cumini*.

INTRODUCTION

Diabetes is one of the chronic diseases that happens when insulin is not produced by the pancreas sufficiently or when the body can not use the insulin effectively.¹ Based on data from the International Diabetes Federation (IDF), it is estimated that 537 million people will have diabetes in 2021. This number will keep increasing in the future, getting to 643 million by 2030 and 783 million in 2045.²

Some drugs used to treat diabetes are alpha-glucosidase inhibitors (acarbose, miglitol, voglibose) and DPP-IV inhibitors (sitagliptin, saxagliptin, vildagliptin, linagliptin, alogliptin). The mechanism of alpha-glucosidase inhibitors is to inhibit alpha-glucosidase enzymes competitively in the small intestine where the dietary starch is digested. Polysaccharide reabsorption and sucrose metabolism will be postponed by inhibiting the enzyme, preventing a significant rise in sugar after eating. Meanwhile, DPP-IV inhibitors inhibit the enzyme dipeptidyl peptidase 4 (DPP-IV), which deactivate the glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). The inhibition of DPP-IV will maintain the inactive form of endogenous GLP-1 and a longer half-life. But, these drugs have side effects, such as diarrhea, abdominal pain, hypoglycemia, nasopharyngitis and pancreatitis.^{3,4}

Because of the side effects of drugs, most diabetic patients consume herbal or traditional medicines, either combined with medications or herbal

alone. They consider that herbal is safer than drugs and believe it can also reduce the side effects caused by drugs. Plants that have been shown to have antidiabetic activity are *Caesalpinia sappan*, *Andrographis paniculata* and *Syzygium cumini*.

Syzygium cumini (synonym: *Syzygium jambolanum* or *Eugenia cumini*) is an original plant from India and the Asian sub-continent. Ayurvedic and Indian Folk Medicine have elaborated the use of this plant for diabetic treatment before the advent of insulin.⁵ Based on the biochemical screening of methanolic and aqueous leaves extracts of *Syzygium cumini*, it showed that those extracts contained alkaloids, saponins, carbohydrates, flavonoids, terpenoids, glycosides and proteins.⁶ Moreover, four types of flavonoid compounds are present in leaf extract of *Syzygium cumini*, quercetin, rutin, ellagic acid and kaempferol. The ethanolic leaf extract showed the highest quantity of quercetin.⁷

Caesalpinia sappan L., commonly known as sappan wood or heartwood, is a plant of the *Leguminosae* family with various medicinal properties, such as skin rashes and excessive body heat, heartburn and indigestion, blood purifier, and diabetes. The pharmacological activity of sappan wood has been proven in various studies, including antioxidant, antibacterial, anti-acne, anti-inflammatory, antihypoglycemic and hepatoprotective. The most abundant water-soluble flavonoid in heartwood is Brazilin which gives red color in the extract. Therefore, sappan wood is also used as a coloring agent for the food, beverages, or textile industry.⁸

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Andrographis paniculata or sambiloto is known as "King of Bitter" because of its bitter taste. The bitter taste comes from compounds named andrographolide and kalmegin 4-lactoneschuanxinlian. Andrographolide is the main bioactive compound found in various parts, especially leaves. Traditionally and pharmacologically, it has been shown to have antidiabetic activity.^{9,10}

Previous *in vivo* studies showed that after seven days, the rats treated by combined extract (1:1:1) with a dose of 150 mg/kg body weight significantly reduced levels of fasting blood sugar, cholesterol levels, and low-density lipoprotein (LDL) levels.¹¹ Some previous studies also had worked on *in vitro* assay. Based on those studies, all extracts have potential antidiabetic activity as an alpha-glucosidase inhibitor. However, not all extracts have data regarding the DPP-IV inhibitory activity. Therefore, it is necessary to determine alpha-glucosidase inhibition and DPP-IV inhibition activity for each extract and combined extract.

MATERIALS AND METHODS

Plant material

Samples used in this study were the aerial part of *Andrographis paniculata* and the wood of *Caesalpinia sappan* collected from Karanganyar-Surakarta, Central Java, Indonesia. The leaves of *Syzygium cumini* were collected from LIPI, West Java, Indonesia. These samples were authenticated by the Indonesian Institute of Sciences (voucher number: B-809/IPH.3/KS/VII/2020).

Chemical

Chemicals used in this study were DPP-IV Assay kit (Cayman Chemical, United States), phosphate buffer pH 6.8, α -glucosidase (Wako Pure Chemical, Japan), para-nitrophenyl-alpha-D-glucopyranoside (pNPG) substrate (Wako Pure Chemical, Japan), acarbose (Sigma, Singapore), dimethyl sulphoxide (Merck, Germany), sodium carbonate (Merck, Germany).

Extraction

The extraction process was the same as the previous study¹¹ and was done by LIPI. Each 500 g of dried powder of *Andrographis paniculata* herbs, *Syzygium cumini* leaves and *Caesalpinia sappan* wood was extracted by maceration process using 70% ethanol. The ratio of dried powder and solvent was 1:10 (w/v). The extract process was done at room temperature for 24-hours. The filtrate was collected, and the residue was re-macerated twice. All filtrate was collected, concentrated with a vacuum rotary evaporator and dried to obtain the dry extract.

LC-MS analysis

The LC-MS analysis of combined extract was conducted by National Research and Innovation Institute, Serpong. The LC-MS analysis was using Xevo G2-XS QToF from Waters (USA). The MS ionization source used ESI (+). The gradient elution system was carried out at a 0.3 mL/min flow rate with an injection volume of 1.0 μ L. The mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) with the following gradient program (v/v): 95% A at 0–1 min, 95% A \rightarrow 0% A at 1.0–11.0 min, 0% A at 11.0–14.0 min, 0% A \rightarrow 95% A at 14.0–17.0 min. The m/z range used from 100.00–1200.00. The energies used for ionization were 6, 10, and 40 eV. The column temperature and sample temperature were maintained at 40 °C and 20 °C, respectively. Other conditions were as follows: capillary voltage of 2.0 kV, desolvation temperature of 550 °C, sample cone voltage of 30 V, extraction cone voltage of 30 V, source temperature of 120 °C, cone gas flow of 50 L/h and desolvation gas flow of 1000 L/h.

Alpha-glucosidase inhibitory activity assay

The alpha-glucosidase inhibitor assay was adapted from Af-idah.¹² Each extract was dissolved in 4% DMSO and phosphate buffer pH 6.8. The combined extract was made by mixture each extract solution in a volume ratio of 1:1:1. Acarbose was used as a positive control. Various concentrations of sample and acarbose were made to determine IC₅₀ of alpha-glucosidase inhibitor. 36 μ L of phosphate buffer, 30 μ L of the sample, and 17 μ L of 3 mM pNPG substrate were put into 96-well microplates. The mixture was incubated at 37°C for 5 min. After incubation, 17 μ L of alpha-glucosidase enzyme 0.025 U/mL were added to each well. The mixture was incubated one more time at 37°C for 15 min to get a complete hydrolysis reaction. After 15 min, 100 μ L of sodium carbonate 200 mM were added to stop the reaction. The absorbance of the samples was measured at 405 nm using a microplate reader (Biochrom EZ Read 400, United Kingdom). Each sample was repeated three times. The summary of pipetting is depicted in Table 1.

The calculation of samples percent inhibition was determined using the following equation:

$$\text{Inhibition (\%)} = [(A_0 - A_1) / A_0] \times 100\%$$

A₀ : absorbance of blanko - control of blanko

A₁ : absorbance of sample - control of sample

The percentage inhibition of each sample showed in a graph, which x as concentration and y as percent inhibition of sample. The graph obtained linear regression equation Y= ax + b to calculate IC₅₀. The IC₅₀ was calculated using following equation : IC₅₀ = (50-a)/b.

DPP-IV inhibitor activity assay

DPP-IV inhibitor assay was tested using the Cayman DPP-IV Assay kit protocol. Sitagliptin (100 μ M) was used as a positive control inhibitor. 30 μ L of the buffer solution, 10 μ L of enzyme DPP IV, 10 μ L of sample solution (40 ppm), and 50 μ L Gly-Pro-AMC as the substrate were mixed into the well. All the samples in the microplate were incubated for 30 min at 37°C. The fluorescence of each sample was measured using a microplate reader (Promega GloMax® Discover System GM3000, United States) on excitation wavelength 350–360 nm and an emission wavelength 450–465 nm. The summary of pipetting is described in Table 2.

Table 1: Procedure pipetting of alpha-glucosidase inhibitory activity.

Reagent	Volume (μ L)			
	Control of Blanko	Blanko	Control of Sample	Sample
Sample/positive control	-	-	30	30
Phosphate buffer pH 6.8	66	66	36	36
pNPG	17	17	17	17
Incubation at 37°C for 5 min				
α -glucosidase	-	17	-	17
Sodium carbonate	100	-	100	-
Incubation at 37°C for 15 min				
α -glucosidase	17	-	17	-
Sodium carbonate	-	100	-	100

Table 2: Procedure pipetting of DPP-IV inhibitory activity.

Well	Volume (μ L)				
	Buffer	DPP-IV	Solvent	Inhibitor	Substrate
100% Initial activity	30	10	10	-	50
Blanko	40	-	10	-	50
Sample/Sitagliptin	30	10	-	10	50

The percentage of inhibition was calculated using formula:

$$\% \text{Inhibition} = \left[\frac{(\text{initial activity} - \text{inhibitor})}{(\text{initial activity})} \right] \times 100\%$$

RESULTS AND DISCUSSION

LC-MS analysis

Based on LC-MS chromatogram (Figure 1), there were four highest compounds identified in combined extract. The most abundant compound in combined extract was 5,7-Dihydroxy-3-(4'-hydroxybenzyl) chromone which has molecular formula of $C_{16}H_{12}O_5$; molecular weight of 284.06 g/mol; ion peak at (M+H) m/z 285.07; and eluted at 3.90 minutes. According to the structure, this compound belongs to the sappanone-type of homoisoflavonoid which derived from *Caesalpinia sappan*. This result in line with Baldim *et al.* (2017), species from genus *Caesalpinia* produce unique compounds named homoisoflavonoids. The most common class of homoisoflavonoids in the genus *Caesalpinia* is the sappanin-type which presents a 3-benzyl chromanone unit. The diversity of these compounds is associated to a wide variation of substituents, such as hydroxyl, methoxyl, formyl, methyl groups which confer to sappanin-type. *Caesalpinia sappan* is a species which produce the most abundant of homoisoflavonoids types, such as brazilin-, caesalpin-, protosappanin- and sappanin-types. In addition, the ion peak in this research was similar with the journal statement that the ion peak of MS spectrum at [M+H]⁺ m/z 250–350 was homoisoflavonoids.¹³

Homoisoflavonoids are oxygen heterocyclic compounds with two aromatic rings, and an additional carbon between the B and C rings on the isoflavonoid skeleton. This class is rare and unique subclass of flavonoids. Classification of homoisoflavonoid is based on the structure and divided into five groups: sappanin, scillascillin, brazilin, caesalpin and protosappanin types.¹⁴

The second most abundant compound was Protosappanin E1. This compound has molecular formula of $C_{32}H_{26}O_{11}$, molecular weight of 586.14 g/mol in the presence of an ion peak (M+H) m/z 587.15 at 4.53 minutes. This compound was derived from *Caesalpinia sappan* as stated in some literatures. Yang *et al.* (2016) stated that isolated chemical compounds from sappan wood classified into 5 different types: hematoxylin, protosappanins, homoisoflavonoids, sappan chalcones and phenylpropanoids. Hematoxylin include brazilin, brazilin and 3'-O-methylbrazilin; protosappanins consist of protosappanins A and B, 10-O methylprotosappanin, isoprotosappanin B, and

protosappanins E1 and E2 (polymer components of hematoxylin and protosappanin).¹⁵ According to the statement of Helmi *et al.* (2020), sappan wood contains brazilin, brazilide A, tetraacetyl-brazilin, brazilane, 3-O methylbrazilin, neuprotosappanin, protosappanin E1, and hematoxylin.¹⁶ The mixture of protosappanins E-1 and E-2 were isolated as a light reddish-brown powder, which showed a single spot on the silica gel thin layer chromatogram. This mixture after methylation with diazomethane, separates into 2 components as the hexamethyl ether. A mixture of protosappanins E1 and E2 produce brazilin and protosappanin B on reductive cleavage with sodium borohydride in alkaline methanol.¹⁷

The third highest compound was a candidate compound with the molecular formula of $C_{36}H_{38}N_4O_5$. This compound has molecular weight of 606.28 g/mol in the presence of ion peak (M+H) m/z 607.29 at 11.17 minutes. According to the literature, this compound was probably pheophorbide A methyl ester. Norazhar *et al.* (2021) identified Pheophorbide A methyl ester at m/z 607.29 with the molecular formula of $C_{36}H_{38}N_4O_5$ in the leaf extract of *Christia vespertilionis*.¹⁸ On the other hand, Bashir (2017) obtained the same compound from Gumbi gumbi, at m/z 606.28 and eluted at 12,094 minutes.¹⁹ Study on *Piper penangense* extract also obtained a pheophorbide A methyl ester with an ion peak at m/z 607.28.²⁰ This compound is a derivative of chlorophyll degradation in green leaves. Pheophorbide A methyl ester has anticancer activity and is known candidate for photodynamic therapy to kill tumors directly by shutting down tumor blood vessels.¹⁹ However, based on the research of Nkobile *et al.* (2021) pheophorbide A methyl ester isolated from *Amaranthus spp.* has the best alpha-glucosidase inhibitory activity (75.41±0.03%) at 0.5 mg/mL.²¹ Because this compound was found in the leaves, this compound might be appeared from *Syzygium cumini* or *Andrographis paniculata*. This can not be confirmed due to the limited references.

The fourth highest compound was Saurufuran B. This compound has the molecular formula of $C_{20}H_{28}O_3$, molecular weight of 316.20 g/mol in the presence of ion peak at m/z 317.2114 at 5.78 minutes. This compound was originally from *Andrographis paniculata*. This result was similar with Handayani *et al.* (2022) in the study investigating the transformation of *A. paniculata* by *Aspergillus oryzae* K1A. According to this study, LC-MS/MS chromatogram data was obtained for compounds contained in *A. paniculata*, one of them was Saurufuran B at m/z at 317.21 with retention time of 7.81 minutes.²² Saurufuran B1 is a naturally occurring as furanoditerpene. Structurally, this molecule is characterized by the presence of alkyl groups and methyl groups at the

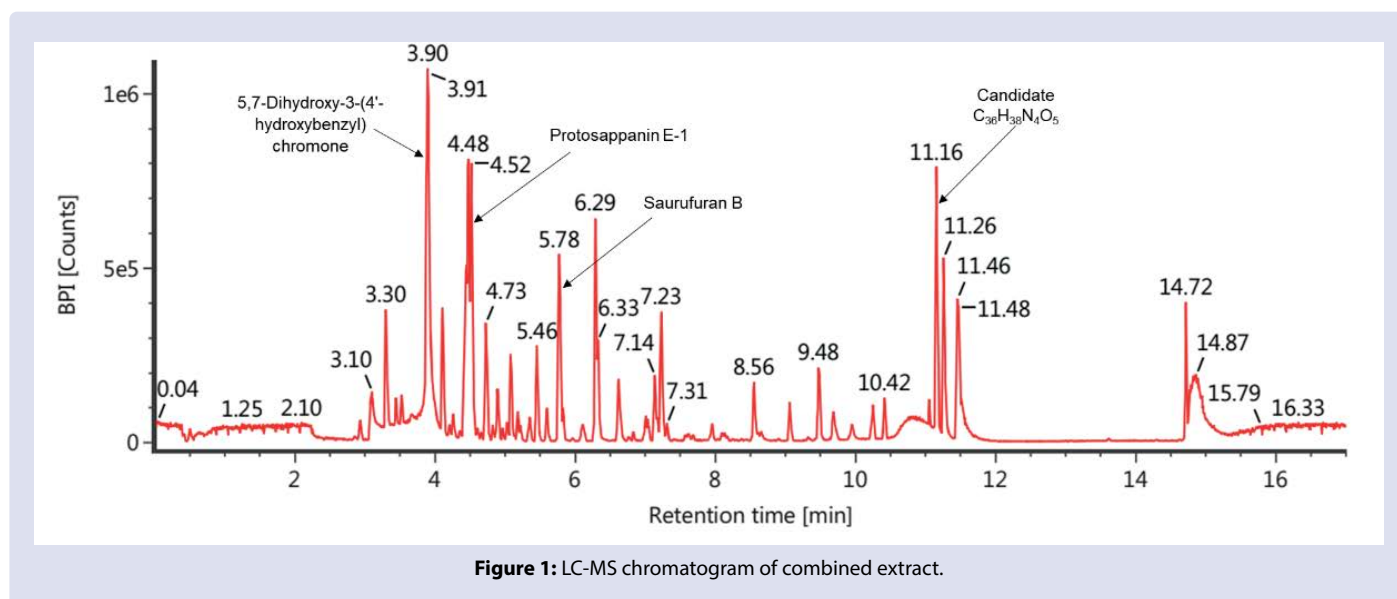


Figure 1: LC-MS chromatogram of combined extract.

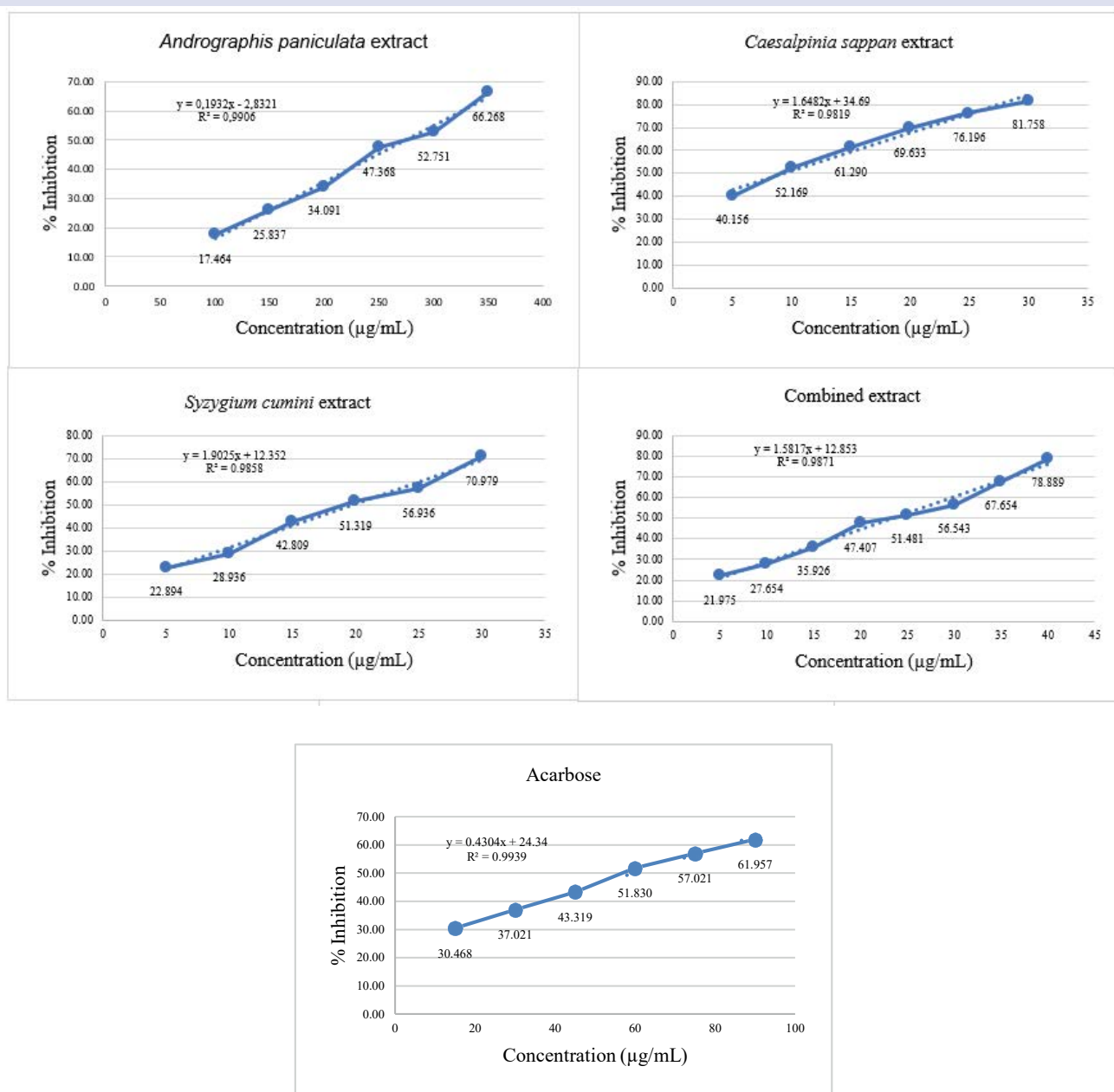


Figure 3: Alpha-glucosidase inhibition of extracts and acarbose.

Table 3: The IC₅₀ value of extract to inhibit alpha-glucosidase.

Samples	IC ₅₀ (µg/mL)
<i>Syzygium cumini</i> extract	19,79
<i>Andrographis paniculata</i> extract	273,46
<i>Caesalpinia sappan</i> extract	9,29
Combined extract	23,49
Acarbose	59,62

Table 4: DPP-IV inhibition of extracts and sitagliptin.

Samples	% Inhibition
<i>Syzygium cumini</i> extract	53,19
<i>Andrographis paniculata</i> extract	33,44
<i>Caesalpinia sappan</i> extract	59,98
Combined extract	63,69
Sitagliptin	99,66

C2 and C3 positions of a furan.²³

Alpha-glucosidase inhibitory activity assay

The assay principle was the inhibitor compound will inhibit the activity of the alpha-glucosidase enzyme from hydrolyzing the pNPG substrate into p-nitrophenol. The hydrolyzed pNPG by the enzyme will change into yellow, and its absorbance was measured spectrophotometrically at a wavelength of 405 nm.

The alpha-glucosidase enzyme inhibitory activity was tested for *Andrographis paniculata* extract, *Syzygium cumini* extract, *Caesalpinia sappan* extract, the combination of three extracts and acarbose as a positive control. All samples used six concentration variations to determine the IC₅₀ value from the regression equation. Calculating the IC₅₀ value can determine the sample ability or strength as an alpha-glucosidase inhibitor. The smaller the IC₅₀ value, the stronger activity of

the sample as an alpha-glucosidase inhibitor. According to categorize of alpha-glucosidase enzyme inhibitory activity, a very strong or potential if the IC_{50} value is $< 50 \mu\text{g/mL}$, strong if the IC_{50} value = $50\text{--}100 \mu\text{g/mL}$, moderate if the IC_{50} value is $100\text{--}150 \mu\text{g/mL}$, weak if IC_{50} is $150\text{--}200 \mu\text{g/mL}$, and very weak if IC_{50} value $> 200 \mu\text{g/mL}$.²⁴

Figure 3 showed that all extracts and acarbose have alpha-glucosidase enzyme inhibitory activity, as seen from the increasing percentage of inhibitory activity as the sample concentration increases. *Caesalpinia sappan* extract obtained the most potent enzyme inhibitory activity with an IC_{50} value of $9.29 \mu\text{g/mL}$ among other extracts, even more potent than acarbose as a positive control with IC_{50} of $59.62 \mu\text{g/mL}$. *Syzygium cumini* extract and combined extract also have potent inhibitory activity with IC_{50} values of 19.79 and $23.49 \mu\text{g/mL}$, respectively. Meanwhile, *Andrographis paniculata* extract has a weak inhibitory activity with an IC_{50} value of $273.46 \mu\text{g/mL}$.

The IC_{50} values obtained for single extracts vary in results compared to previous studies. The results of earlier studies on the ethanolic extract of *Syzygium cumini* leaves obtained an IC_{50} of $26.821 \mu\text{g/mL}$ against the inhibitory activity of the alpha-glucosidase enzyme.²⁵ The 80% ethanol extract of *Caesalpinia sappan* obtained an IC_{50} of $6.3 \pm 0.2 \mu\text{g/mL}$ against alpha-glucosidase inhibitory activity.²⁶ Annamalai et al. (2014) showed different results for the IC_{50} of ethanolic extract of *Caesalpinia sappan*; it was $215.95 \pm 7.52 \mu\text{g/mL}$.²⁷ Arsinngtyas (2015) performed a study of *Caesalpinia sappan* isolates against maltase inhibitory activity. It showed that sappanchalcone and protosappanin B have higher maltase inhibitory activity than brazilin and protosappanin C. But, brazilin and sappanchalcone showed significant glucose uptake enhancement.²⁸ Meanwhile, the study of Subramanian et al. (2008) showed that IC_{50} of ethanol extract of *Andrographis paniculata* was $17.2 \pm 0.15 \text{ mg/mL}$ to inhibit the alpha-glucosidase enzyme. As the biomarker of this plant, andrographolide was tested, and the IC_{50} was $11.0 \pm 0.28 \text{ mg/mL}$.²⁹ On the other study³⁰ showed IC_{50} of *Andrographis paniculata* and andrographolide better than Subramanian et al. According to those studies, it proves that andrographolide has antidiabetic activity as an alpha-glucosidase inhibitor. The IC_{50} of *Syzygium cumini* extract and *Caesalpinia sappan* extract did not make much difference, while the IC_{50} of *Andrographis paniculata* extract obtained better results than previous studies. The difference in the IC_{50} outcome can be affected by several factors, the growth place, harvest, and post-harvest processes, extract methods and solvents used for the extraction process.

DPP-IV inhibitor activity assay

The DPP-IV inhibitor activity of the extract was determined based on the levels of free AMC groups by measuring its fluorescence at $350\text{--}360 \text{ nm}$ as excitation wavelength and $450\text{--}465 \text{ nm}$ as emission wavelength using a microplate reader.³¹ The inhibitory activity of the DPP-IV enzyme was tested on all single extracts ($40 \mu\text{g/mL}$), combined extract ($40 \mu\text{g/mL}$) and sitagliptin ($100 \mu\text{M}$) as a positive control.

The test results showed that the combined extract has the highest inhibitory activity of the DPP-IV enzyme among other extract samples. However, this result was not strong compared to sitagliptin as a positive control that received inhibitory activity of 99.66% . The effects of the inhibitory activity of *Syzygium cumini* extract and *Caesalpinia sappan* extract were not significantly different, 53.19% and 59.98% , respectively. Meanwhile, *Andrographis paniculata* extract gave the lowest inhibitory activity, 33.44% .

In a previous study, the ethanolic extract of *Caesalpinia sappan* (100 ppm) showed 84.25% for DPP-IV inhibitory activity, slightly different from Sitagliptin at 85% . Brazilin, the active compound of *Caesalpinia sappan*, obtained more than. There are some possible mechanisms of brazilin to inhibit the DPP-IV enzyme. Brazilin can increase lipogenesis rate glucose oxidation, regulate 78% inhibition enzymatic processes

in glucose metabolism, decrease serine phosphorylation, stimulate glucose transport and decrease gluconeogenesis in hepatocytes.³² This study proved that brazilin, the most abundant compound in *Caesalpinia sappan*, was responsible for inhibiting the DPP-IV enzyme. On the other hand, the inhibitory activity of the ethanol extract of *Andrographis paniculata* obtained a mark of 37.03% at a concentration of 2.5 ug/mL .³³ For *Syzygium cumini* extract, the seed is the most used to test DPP-IV enzyme inhibitory activity. However, the *Syzygium cumini* leaves as DPP-IV enzyme inhibitors are potential in this study. The quercetin contained in *Syzygium cumini* leaves inhibits the enzyme activity. Quercetin was shown to obtain an IC_{50} of 4.02 nmol/mL against the inhibitory activity of the DPP-IV enzyme. This result was better than sitagliptin with an IC_{50} of 5.49 nmol/mL . Structurally, quercetin interacts with the conformers and forms 5 hydrogen bonds with the hydroxyl groups Val 738, Ser 720, Tyr 700, Ala 732 and Met 733. At the same time, Sitagliptin interacts with the conformers and forms 2 hydrogen bonds with the five-membered nitrogen ring system Histidine 754 and alanine 732. Therefore, the ability to bind more hydrogen, quercetin compound is more potent than sitagliptin.³⁴ On the other hand, myricetin, the most abundant flavonoid obtained from *S.cumini* leaf,³⁵ also has potential to inhibit DPP-IV enzyme with an IC_{50} value of $4.8 \pm 0.36 \mu\text{M}$.³⁶ Myricetin from *S. cumini* showed better affinity to DPP-IV than quercetin. The target proteins of DPP-IV involved in the interaction myricetin, Ser209A, Glu206A, Arg669A, Tyr547A, Tyr666A, Glu205A, Tyr631A, Tyr662A, Phe357A, VaQ11A, Arg125A, Ser630A, Asn710A and His740A.³⁷

CONCLUSION

In conclusion, all extracts have potency as antidiabetic. However, *Caesalpinia sappan* extract had the most vigorous activity among other single extract, either as an alpha-glucosidase inhibitor or DPP-IV inhibitor. Therefore, *Caesalpinia sappan* might have a significant effect on those inhibitor activities. *Caesalpinia sappan* also showed better activity as an alpha-glucosidase inhibitor than acarbose. Meanwhile, the combined extract obtained higher inhibition as a DPP-IV inhibitor than a single extract.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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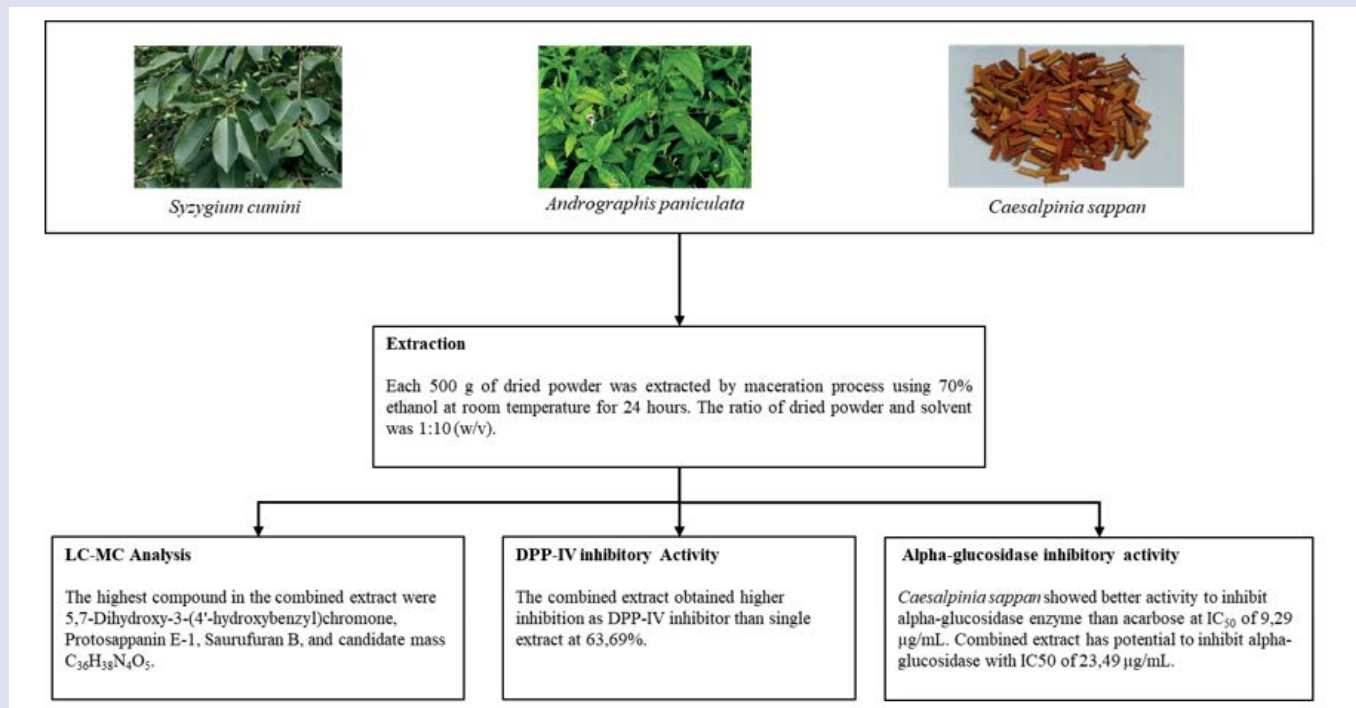
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GRAPHICAL ABSTRACT



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