

Antidiabetic Activity of the Methanol Fraction of Sungkai Leaves (*Peronema canescens* Jack)

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ABSTRACT

Diabetes Mellitus (DM) is one of a group of metabolic diseases characterized by hyperglycemia caused by a disturbance in insulin so that blood glucose levels increase. Prevention of absorption of blood glucose by the intestine can be done with the help of the enzyme α -glucosidase. Based on the results of phytochemical tests, it is known that the methanol fraction of sungkai leaves contains flavonoid compounds. The Fourier Transform Infra-Red (FTIR) spectrum showed that the isolates had phenolic O-H groups ($3,354.53\text{ cm}^{-1}$, $1,359.28\text{ cm}^{-1}$), aromatic C=C group ($1,615.48\text{ cm}^{-1}$), C-O-C ether group ($1,046.60\text{ cm}^{-1}$) and aromatic C-H group (822.21 cm^{-1}). In vivo antidiabetic activity test was carried out using test animals of white male mice which were induced by alloxan. Antidiabetic testing was carried out using 6 treatment groups with glibenclamide, Na-Carboxymethyl cellulose (Na-CMC) 0.5%, dose 175 mg/kgBW, 350 mg/kgBW, 700 mg/kg Body Weight (BW) and isolates 2 mg/kgBW. The results showed that the methanol fraction of sungkai leaves had the best antidiabetic activity at a dose of isolate and 700 mg/kgBW which was able to reduce blood glucose levels by 42.20% and 42.00%. In vitro antidiabetic testing through α -glucosidase enzyme inhibition mechanism did not show any antidiabetic activity at concentrations of fractions 5, 10, 25, 50 and 100 ppm.

Keywords: Diabetes Mellitus, Antidiabetic, Enzyme α -Glucosidase, Sungkai Leaves, Flavonoid.

INTRODUCTION

Diabetes Mellitus (DM) is one of a group of metabolic diseases characterized by hyperglycemia or glucose (blood glucose) levels that exceed normal [1], which is about 200 mg/dl, and fasting blood glucose levels of more than 126 mg/dl caused by abnormalities in insulin, insulin action or both [2]. Diabetes Mellitus is a chronic disease that is often referred to as the silent killer, which is often not realized by the sufferer so that it is detected when complications occur that attack almost all systems of the human body. Diabetes mellitus is one of the non-communicable diseases that occupies the sixth highest position in Indonesia as a disease that causes death [3].

Patients with diabetes mellitus will experience disturbances in insulin which can cause blood glucose levels to increase or hyperglycemia [4]. Prevention of glucose absorption by the small intestine can help prevent an increase in blood glucose. There are several enzymes responsible for the supply of glucose found in the small intestine, one of them is the α -glucosidase enzyme [5]. Several attempts have been made to overcome this diabetes mellitus, namely by doing insulin therapy and can also use chemical drugs so that it can stimulate the

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pancreas. However, many Indonesians have now turned to alternative medicine using medicinal plants. One of the medicinal plants in Indonesia is sungkai (*Peronema canescens* Jack), sungkai leaves are the part that is widely used as medicine. Traditionally, sungkai leaves are used as a cold and fever medicine by the community [6]. Boiled water from sungkai leaves can also be used as a ringworm remedy and mouthwash to treat dental infections [7]. In addition, sungkai leaves also have antiparasitoid activity that can inhibit the growth of parasitemia [8] and has antibacterial activity [9]. *Peronema canescens* (*P. canescens*) can also be used as a natural pesticide that can kill *Artemia salina* Leach larvae [10]. *P. canescens* leaves have potential as antimalarial drugs and has very strong antioxidant activity with an IC₅₀ value of 44.933 ppm [11], can improve health with immunomodulator activity [12] has anticholesterol activity with IC₅₀ value of 60.64 ppm [13]. Based on the previous results of the study that the antidiabetic activity of the ethanolic extract of sungkai leaves was seen starting from day 10 it was able to reduce blood glucose levels of male white mice using doses of 175 mg/kg BW and 350 mg/kgBW. This is comparable to the administration of glibenclamide 3 mg/kg body weight [14].

Based on the results of the phytochemical test, the positive *P. canescens* leaves methanol extract contains alkaloids, flavonoids, glycosides, terpenoids, steroids, and phenolics [15]. Compounds that have antidiabetic activity are flavonoids because they are able to regenerate cells in the islets of Langerhans, thereby increasing the insulin produced and suppressing the amount of blood glucose that enters the cells [16]. With the content of secondary metabolites of the flavonoid group, it is estimated that the methanol fraction of sungkai leaves has antidiabetic activity.

EXPERIMENT

Chemicals and instrumentation

The materials used in this study were Sungkai leaves (*Peronema canescens* Jack), methanol, ethyl acetate (Merck, Darmstadt, Germany), n-hexane, 2 N sulfuric acid, Dragendorff's reagent, Wagner's reagent, Meyer's reagent, Lieberman-Burchard reagent, concentrated HCl, Mg powder, 2N HCl, FeCl₃, anhydrous acetic acid, silica gel, alloxan, 0.5% Na-CMC, glibenclamide, distilled water, sucrose, α -glucosidase enzyme, phosphate buffer, p-nitrophenyl- α -D-glucopyranoside (PNPG), sodium carbonate (Sigma, St. Louis, MI, USA), and male white mice as test animals (Ethical clearance number; 3454/UN21.9/PT/01/04/2022 by Faculty of Medicine and Health Sciences, of Universitas Jambi

The equipment used in this study was a vacuum rotary evaporator, column (Pyrex), capillary tube, test tube, dropper, KBr pellet, measuring flask, vial, stirring rod, watch glass, paper label, 1 mL injection syringe (One Med), 5 mL injection syringe (One Med), 3 mL injection syringe (One Med) analytical balance, animal scale, test animal cage, glucometers, microplate 96 well, microplate reader, UV-Vis Spectrophotometer (Thermo-Fisher Orion Scientific AQ8100, Waltham, MA, USA), FTIR Spectrophotometer (Bruker Alpha II, Germany), and ELISA kit (Thermo Fischer Scientific Varioskan Flash, Madison, WI, USA).

Sample collection and preparation

The sample used in this study was *P. canescens* leaves, was obtained from Koto Baru Hiang Village, Sitinjau Laut Subdistrict, Kerinci Regency, Jambi Province. Sungkai leaves samples were collected and cleaned. Sungkai leaves was then cut into small pieces and dried at room temperature in an open space that was not exposed to direct sunlight [17].

Extraction

P. canescens leaves of as much as 1 Kg were macerated in stages starting in n-hexane solvent for 2 x 24 hours. The maserate was obtained by filtering using Whatman paper No.41 (duplo). The obtained macerate was evaporated using a low-pressure steamer to obtain a thick extract. The rest of the samples were macerated again using ethyl acetate solvent and methanol solvent in the same way. The yield obtained was calculated by weight percentage (w/w) between the yield and the weight of the simplicial powder used by weighing.

Phytochemical screening

Alkaloids

The alkaloid test was carried out with 1 mL of the sample dissolved in a few drops of 2 N sulfuric acid. To test the sample, three types of reagents were used, namely Dragendorff's reagent, Meyer's reagent and Wagner's reagent. If a yellowish white precipitate is formed in the sample with Meyer's reagent, a brown precipitate with Wagner's reagent and a red to orange precipitate with Dragendorff's reagent indicates a positive test result.

Flavonoids

The sample to be tested is added with a few drops of concentrated HCl and added with Mg powder. The test result is positive if there is a change in the color of the solution to orange and foam is formed.

Saponins

To test the presence of saponins can be done by testing foam in hot water. The test results were declared positive if the foam produced was stable for 30 minutes and when 1 drop of 2N HCl was added it did not disappear.

Phenolic

The sample to be tested was added with FeCl_3 and homogenized. If a blackish purple color is formed in the mixture, it indicates that there are phenolic compounds in the sample being tested.

Triterpenoids

The addition of anhydrous acetic acid and concentrated sulfuric acid as Liebermann-Burchard reagents in the sample which shows the formation of a purple or red color then changes to a blue purple or blue green color indicates that there are triterpenoid/steroid compounds in the sample.

Compound separation and purification

Thin layer chromatography (TLC)

TLC plates measuring 1x5 cm were prepared with a lower and an upper limit of 1 cm and 0.5 m, respectively, in order that eluent traveled a distance of 3.5 cm. The eluent was made by comparing organic solvents based on their polarity. The extract was spotted using a capillary tube on the lower boundary of the plate, elution was carried out using the mobile phase. After the mobile phase reaches the upper limit of the plate, the elution process is stopped. Then the stain was examined directly under a UV lamp with a wavelength of 254 nm. All fractions were tested by TLC, fractions with the same spot stain were combined and analyzed by TLC.

Liquid vacuum chromatography

LVC was performed using silica gel as the stationary phase with a sample:silica gel ratio (1:20). Impregnation of the sample extract was carried out using silica gel. Added to the column that already contains the stationary phase. While the mobile phase used is a mobile phase with a gradient polarity. The resulting fraction was then accommodated in vials, the eluate was accommodated based on each band obtained and then evaporated. The results of the column chromatography were carried out by TLC again. The R_f values in the chromatograms was ombined if they have identical stains.

Isolation of Sungkai leaves methanol fraction compound

A 15 g sample was used which was impregnated using 15 g of coarse silica gel (50-10 mesh). Furthermore, fine silica measuring ± 200 mesh was used as packing silica as much as 40 g which was previously oven-dried at 110°C for 15 min to activate the silica and remove water content and increase its reactivity. This silica gel functions as a stationary phase in the KVC process. This separation process was carried out using a column with a diameter of 5 cm and the mobile phase used is a solvent with a gradient polarity. Based on the separation using KVC obtained 32 vials of storage results. The vial was then evaporated at room temperature and the TLC test was carried out with the aim of uniting vials that have the same stain pattern. Based on the TLC results, 3 fractions were obtained.

Characterization of isolated compounds with FT-IR Spectrophotometer

A total of 0.2 g of KBr pellets was added with 1 drop of isolate, then dried and analysed using an FT-IR spectrophotometer at a wave numbers of 4000-400 cm⁻¹ [17].

Table 1. Antidiabetic activity test treatment groups

Groups	Treatment
C-	Alloxan 150 mg/kg bw and Na-CMC 0.5%
C+	Alloxan dose 150 mg/kg bw and glibenclamide 3 mg/kg BW
ME1	Alloxan dose 150 mg/kg bw and sungkai leaf extract 175 mg/kg BW
ME2	Alloxan dose 150 mg/kg bw and sungkai leaf extract 350 mg/kg BW
ME3	Alloxan dose 150 mg/kg bw and sungkai leaf extract 700 mg/kg BW
MI	Alloxan dose 150 mg/kg bw and isolate MeOH fraction of sungkai leaves 2 mg/kgBW

Antidiabetic activity test *in vivo*

Antidiabetic testing begins with induction of mice that have previously been acclimatized for ± 7 days, first measuring the blood glucose levels of mice (H₁). Furthermore, induction using alloxan in mice orally using a dose of 150 mg/kg bw mice in all treatment groups. After induction, mice were given food and drink. The condition of the mice was observed on the sixth day after induction (H₄) and the blood glucose levels of the mice were measured. Diabetic mice with blood glucose levels >176 mg/dL were used as test animals. The next step was for mice to be given a test solution (suspension fraction and glibenclamide) which

was adjusted to the group and the respective dose which lasted for 10 days. On the 10th day after the administration of the test solution (H₁₄), the blood glucose levels of the mice were determined by taking blood from the veins located at the tail end of the mice and then measuring blood glucose levels with a glucometer. When taking blood, the mice must first be fasted for 8 to 12 hours by continuously drinking water [14]. The following was the division of the test animal groups as in Table 1.

α -Glucosidase inhibition test

The sample solution was made in several concentrations dissolved in a phosphate buffer solution of pH 6.8 with acarbose as a comparison. A total of 60 L of the test sample was added with 50 L of 0.1 M phosphate buffer pH 6.8 -glucosidase enzyme was added as much as 1 mL to each well and then incubated at 37 °C for 20 minutes in a 96 well micro plate. Blanks were prepared by replacing 60 L of the test sample with phosphate buffer solution. Prior to incubation, 50 L of 5 mM p-nitrophenyl- α -D-glucopyranose (PNPG) was put into a micro plate followed by incubation for 20 minutes at 37°C. As a final step, the reaction was stopped by adding 160 L of 0.2 M Na₂CO₃ solution into the well and the adsorbent was read at a wavelength of 425 nm with a microplate reader.

RESULT AND DISCUSSION

Phytochemical screening

Phytochemical screening was performed on the obtained viscous methanol extract. The results of phytochemical screening can be seen in Table 2. Based on the result data presented in Table 2, it is observed that the methanol extract of sungkai leaves contains secondary metabolites of flavonoid, phenolic/tanin and saponin groups. Meanwhile, secondary metabolites of alkaloids, steroids and terpenoids showed negative results. Phytochemical screening aims to determine the content of the secondary metabolite group contained in an extract [18].

Table 2. Results of phytochemical screening of *P. canescens* methanol extract

Secondary Metabolites	Results		
	n-Hexane	Ethyl acetate	Methanol
Alkaloids	+	-	-
Flavonoids	-	+	+
Phenolic/Tannin	-	+	+
Saponin	-	-	+
Steroids	-	+	-
Terpenoids	-	-	-

Isolation of *P. canescens* methanol fraction compound

Based on the results of phytochemical screening, the methanol fraction of *P. canescens* leaves is known to contain flavonoid compounds. The flavonoid group compounds have antidiabetic activity, where some of the flavonoid compounds found in the form of glycosides have sugar groups such as amygladin which can capture hydroxyl radicals and prevent diabetes [19]. The methanol fraction of *P. canescens* leaves was isolated to obtain flavonoid compounds using Liquid Vacuum Chromatography (LVC). Prior to the LVC process, Thin Layer

Chromatography (TLC) was carried out which aims to find the best mobile phase to be used in column chromatography. The three fractions were subjected to phytochemical screening to test the compounds contained in the fractions obtained from the LVC results. Based on the results of phytochemical screening, the three methanol fractions of sungkai leaves were positive for secondary metabolites of the flavonoid group. Furthermore, the isolates that had been obtained were subjected to a phytochemical screening test to determine the content of secondary metabolites in the isolates. Pure isolates 1 and 2 have not been obtained, thus further purification is needed. The purification results of fraction 3 (isolate) were analyzed by phytochemical screening (Table 4).

Table 3. Phytochemical screening results of LVC fractions

Secondary Metabolites	F1	F2	F3
Alkaloids	-	-	-
Flavonoids	+	+	+
Phenolic	-	-	-
Saponins	-	-	-
Steroids	-	-	-
Terpenoids	-	-	-

Table 4. Results phytochemical screening for F3 isolate

Secondary Metabolites	Results
Alkaloids	-
Flavonoids	+
Phenolic/Tanin	-
Saponin	-
Steroid	-
Terpenoid	-

Characterization of isolated compounds using FTIR Spectrophotometry

FTIR spectrophotometry was carried out with the aim of identifying the functional groups present in a compound based on the difference in the absorption of wave numbers.

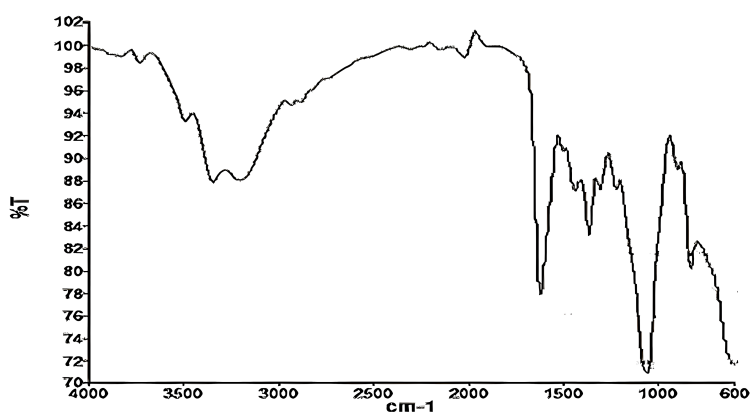


Figure 1. FTIR Spectra of isolate F3

Based on the FTIR spectra of isolate F3 from the methanol fraction of sungkai leaves, there is a wide band that correspond to the absorption of O-H at 3354.11 cm^{-1} . Peaks at 1615.48 cm^{-1} indicates the presence of an aromatic C=C functional group. Absorption at 1359.28 cm^{-1} suggests the O-H functional group of phenol. The stretching vibration of C-O functional group was shown at 1046.60 cm^{-1} . Absorption of the C-H groups was observed in the area of 2900-2800 cm^{-1} . The infrared spectra of isolate F3 have similarities with that of reported in previous studies, which suggest the presence of quercetin compounds [20].

Table 5. Tabulation of FTIR spectra isolate and quercetin

Isolate F3	Quercetin [20]	Functional Groups
3,354.53	3,290.58	O-H phenol
1,359.28	1,359.47	
1,615.48	1,612.16	C=C aromatic
1,046.60	1,163.60	C-O-C ether
822.21	816.46	C-H aromatic

The results of characterization using FTIR, it is most likely that the F3 isolate of the methanol fraction of Sungkai leaves contains secondary metabolites of the flavonoid group. It can be seen from the functional group of the F3 isolate of the methanol fraction of Sungkai leaves which has a functional group that is identical to the functional group in the flavonoid group compounds. The functional groups identified in flavonoid compounds are hydroxy compounds (OH), carbonyl (C=O), ethers (C-O), alkenes (C=C) and aromatic rings. The wave number confirming the flavonoid compound was read in the 1615.48 cm^{-1} region [21]. This is in accordance with the results of phytochemical screening, that the F3 isolate of the methanol fraction of Sungkai leaves was positive for secondary metabolites of the flavonoid group.

Flavonoid compounds have the ability to lower blood glucose levels. This is because the phenolic and flavonoid group compounds have antihyperglycemic or antidiabetic effects [22]. According to these groups compounds have several possible mechanisms, being able to inhibit glucose absorption in the body, secrete insulin or act like insulin and be able to regulate the activity of enzymes that play a role in carbohydrate metabolism. Flavonoids are also known as antidiabetic compounds because they have the potential to inhibit the glucose transport process in the small intestine so that they can reduce blood glucose levels [23].

Antidiabetic activity test *in vivo*

The *in vivo* studies was conducted using white mice (*Mus musculus*), 30 mice were divided into six treatment groups (5 mice per group). The test animals used white mice that are 2-3 months old with a body weight of about 20-30 grams. Mice were used as test animals because mice are animals with a high reproductive rate with a short life span of about 2-3 years, mice are easy to adapt to new environments, the body structure is easy to understand, and has characteristics similar to human [24]. Meanwhile, the mice used were male due to having a more stable hormonal system when compared to female mice and male mice have a faster drug metabolism rate [25]. The following is a table of average blood glucose levels *in vivo* antidiabetic activity tests.

Based on the results of the treatment, it was found that the decrease in blood glucose levels occurred in the isolate treatment group, a dose of 700 mg/kgBW, glibenclamide 3 mg/kg

BW, a dose of 350 mg/kg BW, a dose of 175 mg/kgBW and suspension of Na- CMC 0.5%. Prior to alloxan induction, the mice were first measured blood glucose levels, the results ranged from 95-136 mg/dL, which is still normal in this range.

Table 6. Average blood glucose levels from antidiabetic activity test *in vivo*

Treatment Group	Average blood glucose level (mg/dL) \pm SEM			Percentage of blood glucose level
	H ₀	H ₄	H ₁₄	
C+	136 ^a \pm 5.50	183.7 ^a \pm 21	107.3 ^b \pm 10.0	-41.75%
C-	110.7 ^a \pm 4.50	177.0 ^a \pm 15	180.7 ^c \pm 20.0	+2.09%
ME1	95.7 ^a \pm 1.52	156.0 ^a \pm 1.0	109.0 ^b \pm 0.5	-30.13%
ME2	97.3 ^a \pm 0.51	155.7 ^a \pm 1.0	93.5 ^{ab} \pm 14.5	-42.00%
ME3	100.7 ^a \pm 12.01	142.0 ^a \pm 11.5	83.0 ^a \pm 0.5	-41.34%
MI	99.7 ^a \pm 3.01	155.7 ^a \pm 7.0	92.0 ^{ab} \pm 3.5	-42.20%

The antidiabetic effect provided by the methanol fraction of *P. canescens* leaves is supported by the results of previous studies which state that *P. canescens* leaves contain flavonoid secondary metabolites [17]. Flavonoid and phenolic compounds have a linear effect on antidiabetic activity, where the higher the level, the better the antidiabetic activity. Flavonoids have an antidiabetic effect through several mechanisms, namely by inhibiting the absorption of glucose in the body, being able to stimulate insulin secretion or being able to act according to insulin and being able to regulate the performance of enzymes that play a role in the carbohydrate metabolism process [26].

One way ANOVA test was performed with a significance value obtained ($p > 0.05$) which stated that there was no significant difference between each treatment group before treatment (H₀) because the mice's blood glucose conditions were still within normal limits. Meanwhile, the data on blood glucose levels after alloxan induction (H₄) obtained a significance value ($p > 0.05$), which means that there was no significant difference in each treatment group and after giving the fraction (H₁₄) a significant value was obtained ($p < 0.05$) which means that there are significant differences in each treatment group. Duncan's post hoc follow-up test showed that there were significant differences between each treatment group, which proved that the administration of the methanol fraction of *P. canescens* leaves to male white mice was able to reduce blood glucose levels in diabetic mice.

Table 7. Average changes in body weight of mice

Treatment Group	Average Weight (mg/dL) \pm SEM		
	H ₀	H ₄	H ₁₄
C+	26.45 ^a \pm 2.95	27 ^a \pm 4.40	30.3 ^a \pm 3.10
C-	23.6 ^a \pm 4.00	27 ^a \pm 3.00	26.7 ^a \pm 0.25
ME1	25.3 ^a \pm 3.70	29.7 ^a \pm 0.25	29.0 ^a \pm 0.10
ME2	28.4 ^a \pm 0.10	30.3 ^a \pm 1.00	32.2 ^a \pm 0.25
ME3	25.3 ^a \pm 1.00	28.4 ^a \pm 0.30	29.6 ^a \pm 4.25
MI	24.4 ^a \pm 3.65	26.9 ^a \pm 2.60	33.0 ^a \pm 1.60

Based on normality test and homogeneity test of all treatment groups; before treatment (H₀), after alloxan induction (H₄), and after treatment (H₁₄) had a significant value ($p > 0.05$), which

means the data obtained were homogeneous and normally distributed. A one-way ANOVA analysis shows a significance value ($p > 0.05$), this means that there is no significant difference between each treatment group.

Antidiabetic activity test *in vivo*

This test was carried out by inhibiting the action of the α -glucosidase enzyme *in vitro* and then the product adsorption from the enzymatic reaction was measured calorimetrically using the ELISA Reader. The highest enzyme activity is given to the use of substrate with the lowest IC_{50} value [27]. Based on the results of the inhibition test of the α -glucosidase enzyme presented in Table 7, it is known that the methanol fraction of *P. canescens* leaves has IC_{50} 431.26 μ g/mL, while the acarbose compound as a comparison compound obtained an IC_{50} value of 0.22 μ g/mL. This means that acarbose has higher antidiabetic activity than methanol extract. Acarbose is a substrate that can inhibit the α -glucosidase enzyme. Acarbose has a similar structure to oligosaccharides. Acarbose can occupy the active site of the α -glucosidase enzyme easily hence it can inhibit the work of the α -glucosidase enzyme [5]. In addition to the mechanism of inhibiting alpha-glucosidase, antidiabetic activity can be analyzed through several different mechanisms, so that several flavonoid compounds can also have different pathways [28].

Table 8. *In vitro* antidiabetic activity test results

Samples	Concentration (ppm)	Absorbance	% Inhibition	IC_{50} (μ g/mL)
Methanol Fraction	100	1.154	12.90	431.26 \pm 0.33
	50	1.103	8.56	
	25	1.093	7.70	
	10	1.063	4.82	
	5	1.016	0.21	
Acarbose	10	0.076	95.90	0.22 \pm 0.77
	5	0.093	93.63	
	1	0.223	72.92	
	0.5	0.303	60.69	
	0.1	0.443	37.79	

Based on the results of *in vitro* antidiabetic activity testing, it is known that the flavonoid group in the methanol fraction of *P. canescens* leaves show a weak ability of antidiabetic activity through the mechanism of α -glucosidase enzyme inhibition. This seems the concentration used in the tested *P. canescens* leaves methanol fraction was not sufficient enough to inhibit the α -glucosidase enzyme. The *in vitro* activity suggests that there may be a possible antidiabetic mechanism other than glucosidase inhibition [29].

CONCLUSION

Our finding that bioactive compounds from the methanol fraction of *P. canescens* leaves were analyzed using an FTIR spectrophotometer, it is suspected that the methanol fraction of sungkai leaves contain flavonoid compounds. The methanol fraction of sungkai leaves can reduce blood glucose levels in mice which are 42.20%; 42.00%; 41.34%; and 30.13% for isolate dose, 350 mg/kgBW, 700 mg/kg bw, and 150 mg/kgBW, respectively.

Moreover, the methanol fraction of sungkai leaves has IC₅₀ 431 inhibiting the alpha-glucosidase enzyme.

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