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In vitro antidiabetic activity of *Peperomia pellucida* extract and fraction by alpha-amylase inhibition pathway

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ABSTRACT

The development of diabetes, especially type 2 diabetes, is influenced by the important role of postprandial blood glucose. One strategy for controlling postprandial hyperglycemia is inhibiting the digestion of dietary carbohydrates through inhibition of alpha-amylase and alpha-glucosidase enzymes. A natural alpha-amylase enzyme inhibition strategy that utilizes natural ingredients is an important alternative in the development of antidiabetic drugs. *Peperomia pellucida* herb is one of the plants which can be potentially be developed as an antidiabetic. This study aims to examine the effects of antidiabetic extract and ethyl acetate fraction of *Peperomia pellucida* in inhibiting the alpha-amylase enzyme. The research was conducted by determining the total flavonoid content using the quercetin standard. Antidiabetic studies were carried out by reducing sugar produced from the hydrolysis starch determined by colorimetric assay with dinitrosalicylic reagent. The activity of extract and fraction of *Peperomia pellucida* in inhibiting alpha-amylase enzyme were analyzed using UV-Vis spectrophotometer instrument. Total flavonoid content was calculated by entering the absorbance value into the standard quercetin curve, while the IC_{50} value of in vitro antidiabetic activity was determined by entering the value 50 on the curve between the sample concentration and % inhibition of the alpha-amylase enzyme. The results showed that the total flavonoid content of the ethanol extract and the ethyl acetate fraction of *Peperomia pellucida* were 88.24±3.07 mg QE/g extract and 80.45±2.81 mg QE/g fraction, respectively. Based on the results, the inhibition of the alpha-amylase enzyme showed that the ethanol extract and the ethyl acetate fraction of *Peperomia pellucida* had an inhibitory activity with the IC_{50} value of the ethanol extract 1066.20 μg/mL and the ethyl acetate fraction 907.19 μg/mL. Meanwhile, the control of acarbose showed an IC_{50} value of 499.96 μg/mL. Ethanol extract and ethyl acetate fraction of *Peperomia pellucida* herbs have an antidiabetic activity with the mechanism of inhibiting the activity of alpha-amylase enzyme, but the activity is lower than acarbose.

Keywords: *Peperomia pellucida*, alpha-amylase, total flavonoid content, ethanol extract, ethyl acetate fraction

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INTRODUCTION

Diabetes mellitus has caused 4.9 million cases of death with a percentage of 90% in the world (Ade loye et al., 2017). The development of diabetes, especially type 2 diabetes, is influenced by the important role of postprandial blood glucose (Alongi & Anese, 2018). One of the strategies which can be employed to control postprandial hyperglycemia is inhibiting the digestion of dietary carbohydrates through inhibition of alpha-amylase and alpha-glucosidase enzymes (Alqahtani et al., 2020; Tundis et al., 2010).

The hydrolysis of starch by alpha-amylase and absorption in the intestines by alpha-glucosidase can result in a sudden increase in blood glucose, causing hyperglycemia in cases of type 2 diabetes mellitus (Ali et al., 2012). Alpha-amylase, which is released from the salivary glands and the pancreas, catalyzes the breakage of alpha-1,4 glycosidic linkages, converting polysaccharides into smaller oligosaccharides like maltose, maltotriose, and a variety of alpha-1,4 and alpha-1,6-oligoglycans. These fragments are also involved in the hydrolysis process, where they are degraded by alpha-glucosidase, an enzyme that catalyzes oligosaccharides and disaccharides to monosaccharides able to be absorbed and entered into the bloodstream (Alagesan et al., 2012). As a result, the inhibition of this enzyme can slow the digestion of the carbohydrates so as to reduce absorption resulting in suppression of postprandial hyperglycemia (Trinh et al., 2016).

Alpha-amylase is the main product secreted by the pancreas (5-6%) and saliva (Sales et al., 2012). This enzyme is bound to the epithelium membrane of the small intestine which serves as a catalyst for the hydrolytic division of oligosaccharides into monosaccharides in the process of glucose absorption in the small intestine. The inhibition process of these enzymes in the intestine will cause the hydrolytic cleavage rate of oligosaccharides to decrease and the carbohydrate digestion. This process occurs in the lower part of the small intestine so that the total glucose rate absorption into the blood is slowed (Shai et al., 2010). A natural alpha-amylase enzyme inhibition strategy that utilizes natural ingredients is an important alternative in the development of antidiabetic drugs. *Peperomia pellucida* herbs is one of the plants which can be potentially be developed as an antidiabetic.

Several studies have shown that the bioactive components in plant extracts are able to inhibit alpha-amylase enzyme (Alqahtani et al., 2020; Daud et al., 2019). In vivo research shows that *Peperomia pellucida* can reduce streptozotocin-induced blood glucose levels in mice (Hidayati, 2021). Research on how the effect reduced blood glucose rate in mice with a sucrose induced model showed that the ethanol extract of *Peperomia pellucida* was capable of lowering it effectively (Salma et al., 2013). Based on this review, *Peperomia pellucida* herbs have the potential to be used as antidiabetic medications. Therefore, it is important to conduct research on the antidiabetic activity of plant extract *Peperomia pellucida* against the inhibited enzyme alpha-amylase.

MATERIALS AND METHOD

Plant material

Herbs of *Peperomia pellucida* were collected in the Curahkalong area of Jember. Determination was conducted in the Biology Laboratory, Faculty of Applied Science and Technology, Ahmad Dahlan University with the number 08/Lab. Bio/B/I/2019. All parts of the plant were taken and cleaned with running water, then cut into ± 2 cm, and aerated until wilted and dry. Then, the drying process was maximized with an oven at a temperature of 40^{o}C until dry and the sample was mashed with a blender.

Chemicals and reagents

Alpha-amylase from *Aspergillus oryzae* (**Sigma-Aldrich**), quercetin and 3,5-dinitrosalicylic (**Sigma-Aldrich**). Ethanol, ethyl acetate, NaOH, $NaH2PO_4$, Na_2HPO_4, KNa-Tartart, $AlCl_3$, potassium acetate, potato starch. The instrument used in this research is the Shimadzu UV-1900i UV-VIS spectrophotometer.

Preparation of plant extracts

Extraction was carried out by taking as much as 100 g of dry powder macerated using 750 mL of 96% ethanol for 1 hour in ultrasonication, then filtered. The macerate was evaporated with a rotary evaporator vacuum at a temperature of 50ºC. Fractionation was carried out by taking two parts of the ethanol extract of the *Peperomia pellucida* herbs and 1 part of aquadest. Furthermore, the mixture was added with two parts of ethyl acetate solvent, shaken in a separatory funnel for 10 minutes, and then allowed to stand until separated into two layers. The upper part of the ethyl acetate fraction was taken and evaporated in a fume hood.

Determination of total flavonoids content

Total flavonoids content was identified by determining the levels of the marker compound quercetin by spectrophotometry. Determination was done by making a series of standard solutions of quercetin with concentrations of 5, 10, 15, 15, 20, and 25 µg/mL. Ethanol extract *Peperomia pellucide* and sample solution ethyl acetate fraction were made by dissolving 20 mg of the sample in 10 mL of ethanol. After that, 100 µl of sample or standard was taken, and 500 µl of ethanol, 200 µl of 1% $AlCl_3$, 200 µl of potassium acetate solution were taken, and then incubated at room temperature for 30 minutes before being measured using spectrophotometry at a wavelength of 432 nm. Quercetin levels were obtained by entering the sample absorbance into the standard quercetin curve, and then the total flavonoid content was calculated in mg QE/g extract (Rosidi et al., 2021).

Alpha-amylase inhibitory assay

Antidiabetic studies were carried out by reducing sugar produced from the hydrolysis starch, which was determined by colorimetric assay with a dinitrosalicylic reagent. The 3,5-dinitrosalicylic acid (DNS) reagent produced a dark orange 3-amino-5-nitrosalicylic acid compound when it reacted with reduced sugar. The alpha-amylase inhibitory activity test refers to the procedure used by (Wulandari et al., 2020) with modifications. A total of 100 µl of sample and positive control of acarbose were pipetted into a test tube, added with 25 µl of enzyme and buffer to 500 µl which was then incubated for 10 minutes at 25ºC. The blank contained the volume of the solution without enzymes. 100 µl potato starch substrate was added to the mix and incubated again at 25ºC for 10 minutes. The cessation of reaction was done by giving DNS 400 µl and heated in boiling water on a hot plate for 15 minutes. Then, its absorbance was measured using UV-Vis spectrophotometer instrument at a wavelength of 540 nm.

Data Analysis

The antidiabetic activity of the sample is seen from the magnitude of the alpha-amylase enzyme inhibition, which can be calculated by:

$$\% \text{ Inhibition} = \frac{\text{Ab Control} - \text{Ab Sample}}{\text{Ab Control}} \times 100\%$$

The IC_{50} value of the sample was determined using the linear regression equation formula between the sample concentration and % inhibition. The IC_{50} was determined using the regression equation.

RESULT AND DISCUSSION

Extraction and fractionation of *Peperomia pellucida* herbs

Peperomia pellucida powder was macerated with 96% ethanol and continued by fractionation using ethyl acetate solvent. The yield of extracts and fractions on the weight of simplicia was presented in Table 1.

Table 1. Percentage of extracts and fractions of *Peperomia pellucida* herbs

Sample	Sample Weight (g)	Yield Weight (g)	Percent Yield (%)
Ethanol extract	946	76	8.03
Ethyl acetate fraction	65	5.55	0.69

Determination of total flavonoid content extract and fraction of *Peperomia pellucida*

The determination of quercetin was carried out using the visible spectrophotometric method. Optimization was carried out to determine the maximum wavelength. The optimization results show that the maximum wavelength is 432 nm. The standard curve was made by making a series of dilutions of the quercetin standard with ethanol as a solvent. The results of the determination of the quercetin standard curve with an R^2 value of 0.9986. are shown in Figure 1.

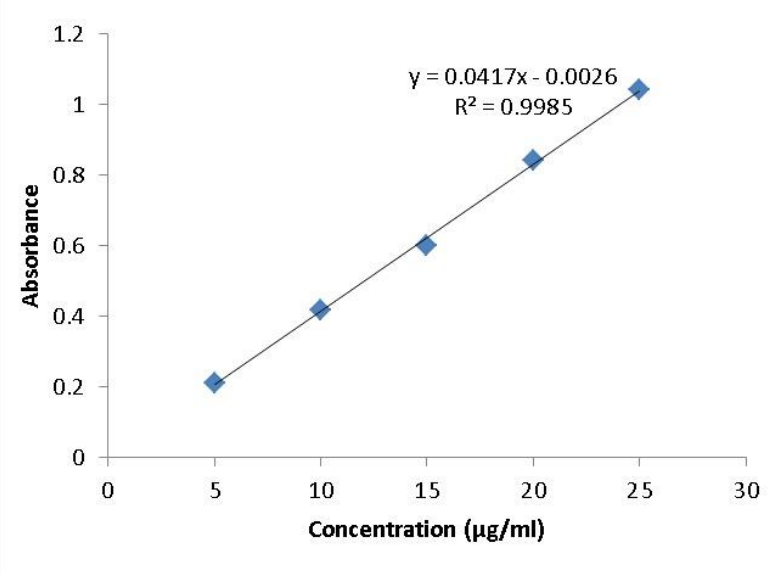

Figure 1. The standard curve of quercetin to determine of total flavonoid content

Quercetin has been known to be soluble in ethanol solvents (Abraham & Acree, 2014; Aguda & Chen, 2016). Furthermore, the total flavonoids content was calculated and the results were obtained, as shown in Table 2.

Table 2. Total flavonoid content extracts and fractions of *Peperomia pellucida*

Sample	Total Flavonoid content (mg QE/g)	Sig.
Ethanol extract	88.24±3.07	0.134
Ethyl acetate fraction	80.45±2.81	

Based on data in Table 2. the results of t-test from the total flavonoid content show the sig. 0.134. So, the value of the total flavonoid content between the ethanol extract and the ethyl acetate fraction has no significant difference. The total flavonoids content in the extract and the fraction of the herb extracts were 88.24±3.07 mg QE/g and 80.45±2.81 mg QE/g respectively. Another study determining the total

flavonoid content of an ethanol extract *Peperomia pellucida* showed a smaller value, which was 31.05±0.35 mg QE/g extract (Sembiring et al., 2018). Quercetin is a flavonoid that can be found in over twenty different plant materials. One of the most extensively utilized flavonoids for the treatment of metabolic and inflammatory illnesses is quercetin (David et al., 2016). Quercetin is an aglicon derivate of flavonoid glycosides produced by plants which has been used as a nutritional supplement and may be effective against various diseases. Quercetin is an important flavonoid, which has many benefits, including lowering blood pressure, antihyperlipidemic, antihyperglycemic, antioxidant, antiviral, anticancer, anti-inflammatory, antimicrobial, neuroprotective, and cardioprotective effects (Hosseini et al., 2021).

The alpha-amylase enzyme inhibitory activity of extracts and fractions

The determination of the antidiabetic activity of the *Peperomia pellucida* herbs was carried out in vitro using the enzyme alpha-amylase inhibition method. The enzyme alpha-amylase is able to hydrolyze starch into reduced sugars. Starch will be hydrolyzed by the alpha-amylase enzyme by breaking the glycosidic-D-(1-4) bond into shorter oligosaccharide reducing sugars. DNS will react by lowering sugar levels to form 3-amino-5-nitrosalicylic acids with a dark orange color and a wavelength of 540 nm (Figure 2). The greater 3-amino-5-nitrosalicylic acid formed, the higher the decrease sugar activity is. Inhibition of the alpha-amylase enzyme will result in a decrease in the absorbance of 3-amino-5-nitrosalicylic acid (Timerman, 2012).

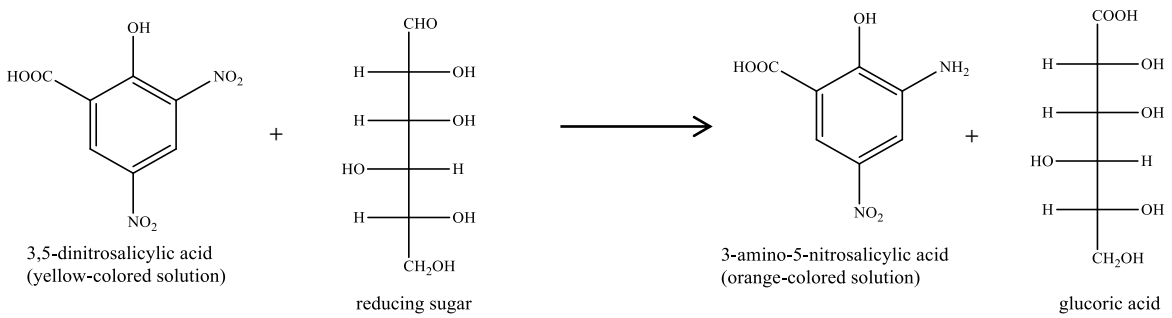

Figure 2. The chemical reaction between DNS and reducing sugar as a result of starch hydrolysis produces 3-amino5-nitrosalicylate with a dark orange color

Based on the linear equation between the sample levels and the percent inhibition of the alpha-amylase enzyme (Figure 3), the IC_{50} value of the herbal extracts against the alpha-amylase enzyme was calculated. The IC_{50} results can be seen in Table 3, where the IC_{50} value of the herbal ethanol extract of *Peperomia pellucida* herbs to the alpha-amylase enzyme is 1066.20 µg/mL and the IC_{50} of the ethyl acetate fraction of *Peperomia pellucida* herbs is 907.19 µg/mL. Meanwhile, the IC_{50} value for comparison of acarbose was smaller than the IC_{50} value of the extract and the fraction of the herbs, which was 499.96 µg/mL. The extract can inhibit the alpha-amylase enzyme due to the content of flavonoid compounds and polyphenols (Moein et al., 2017). In the ethanol extract of the *Peperomia pellucida* herbs, there was a flavonoid quercetin content of 88.24±3.07 mg QE/g extract. Flavonoids are polyphenolic compounds that are polar. So, they tend to dissolve in polar solvents and slightly soluble in semipolar solvents.

Flavonoids have been extensively studied for their wide range of bioactive, including antioxidation, cardioprotection, antibacterial, and anti-inflammatory properties (Wang et al., 2017). Flavonoids are recognized to control the digestion of food in animals interacting with digestive enzymes. The results of the flavonoid docking analysis are closely related to the active site of the enzyme, while acarbose is related to the site behind the catalytic triad. The results of docking analysis and extrinsic fluorescence analysis showed that a hydrophobic interaction regulates flavonoid-alpha amylase

interactions (Martinez-Gonzalez et al., 2019). Inhibition of starch digestion by flavonoids through the formation of flavonoid starch complexes in the process of hydrophobic interactions, and the formation of starches that cannot be digested by alpha-amylase by forming covalent bonds of flavonoids and starches in the cooking and cooking process (Takahama & Hirota, 2018).

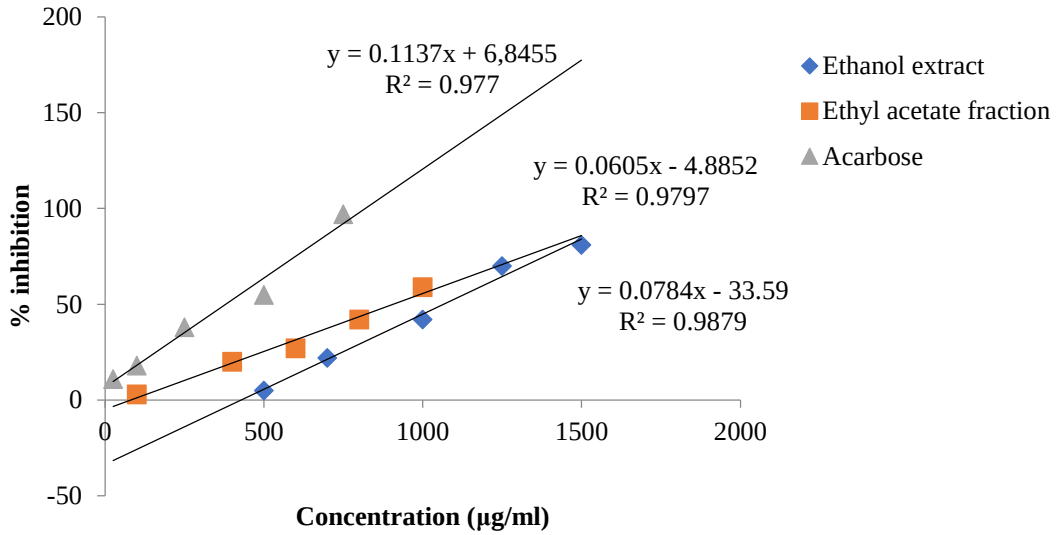

Figure 3. Linear regression curve of ethanol extract, ethyl acetate fraction of *Peperomia pellucida*, and positive control of acarbose with % inhibition to calculate of IC_{50}

Table 3. In vitro activity of *Peperomia pellucida* herbs against alpha-amylase enzyme inhibition

Sample	Ethanol Extract	Ethyl Acetate Fraction	Acarbose
IC_{50} (µg/mL)	1066.20	907.19	499.96

Several studies have proven the activity of flavonoids against alpha-amylase enzyme inhibition. Lutein, myricetin, and quercetin were found to be strong inhibitors of alpha-amylase in studies utilizing porcine pancreatic alpha-amylase (Tadera et al., 2006). Flavonoids have been extensively studied as alpha-amylase inhibitors, which have the potential to be used in the treatment of diabetes (Zhu et al., 2020).

CONCLUSION

Ethanol extract and ethyl acetate fraction of *Peperomia pellucida* herbs have an antidiabetic activity with the mechanism of inhibiting the activity of alpha-amylase enzyme, but the activity is lower than acarbose.

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