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The Potential of Methanol Extract from Avocado Seeds (*Persea Americana* Mill) As an Alfa-Glucosidase Inhibitor

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ABSTRACT

Alpha-glucosidase inhibitors are commonly used as first-line drugs in the treatment of Type 2 Diabetes Mellitus, specifically to manage postprandial hyperglycemia. Avocado seeds (*Persea americana* Mill) have been recognized for their antidiabetic properties, attributed to their rich content of bioactive compounds such as polyphenols, flavonoids, tannins, and alkaloids. The antidiabetic activity of these compounds can be evaluated by measuring the absorption of p-nitrophenol, a product of the hydrolysis of p-nitrophenyl-α-D-glucopyranose (PNP) by the α-glucosidase enzyme. This study aims to assess the antidiabetic potential of methanol extract from avocado seeds by determining its α-glucosidase inhibitory activity based on the IC_{50} value. **Methods.** The methanol extract was obtained through maceration, and its α-glucosidase inhibitory activity was analyzed using an ELISA reader (Enzyme-Linked Immunosorbent Assay) at a wavelength of 405 nm. **Results and Conclusion.** The findings revealed that the methanol extract of avocado seeds exhibited significant inhibitory activity, with an IC50 value of 11.314 µg/mL, classifying it as a highly potent α-glucosidase inhibitor.

A. INTRODUCTION

Glucose is the main source of energy, which is obtained from the carbohydrates consumed. Usually, Insulin is a hormone that converts carbohydrates into glucose, which will be used as fuel in cells as an energy source. However, if the glucose level in the blood doesn't decrease as much as it should, it may indicate a sign of type 2 diabetes. Diabetes mellitus type 2 (T2DM) is characterized by a continuous increase in blood glucose or an increase in blood glucose after eating carbohydrates or postprandial (Michelle, 2024) (Eric, 2021).

The prevalence of T2DM is approximately 90% among global diabetes patients and is expected to reach 592 million by the end of 2035. Hyperglycemia is the most critical criterion of all types of diabetes, and its consistency leads to various complications such as cardiovascular disorders, kidney failure, neuropathy, lipid metabolism disorders, etc. Therefore, controlling blood glucose levels in diabetes patients is of utmost importance (Uday, Abhishek, Sumit, & Parames, 2020).

One therapeutic approach to treating diabetes is to reduce postprandial hyperglycemia. This can be achieved by delaying glucose absorption through the inhibition of the carbohydrate hydrolysis enzyme, alpha-glucosidase, in the digestive tract. Alpha Glucosidase Inhibitors (AGI) reversibly inhibit a number of alpha-glucosidase enzymes (for example, maltase), thereby delaying glucose absorption from the intestine. AGI may be a reasonable choice as a first-line medication for patients with T2DM as it specifically targets postprandial hyperglycemia (Lalitha & Palani, 2015).

One of the plants commonly used for diabetes mellitus is avocado seeds (Persea americana Mill) because they contain saponins, tannins, flavonoids, phenolics, and alkaloids (Nurdin, 2023) (Setyawan, Sukardi, & Puriwangi, 2021). Flavonoids, with hydroxyl groups, play an important role as antidiabetic agents (Murni, et al., 2017). Plants that contain flavonoids have shown beneficial effects on diabetes mellitus by reducing glucose absorption or increasing insulin secretion; furthermore, flavonoids can stimulate glucose absorption in peripheral tissues and regulate the activity of enzymes involved in carbohydrate metabolism (Ervianingsih, Hurria, Astari, Mursyid, & Kadir, 2020).

Several solvents commonly used for the extraction process include water, ethanol, methanol, acetone, and other organic solvents. Each solvent has different capabilities for extracting various phytochemical compounds from samples (Setyawan, Sukardi, & Puriwangi, 2021). Methanol can extract phytochemical compounds more effectively than other solvents such as petroleum ether, ethyl acetate, water, and chloroform (Gangadhara, Valmiki, Radhakrishnan, Geetha, & Ramanathan, 2019) (Ilozue, Ikezu, & Ugwu, 2014). The phytochemical content of the extract significantly influences its biological activity (Ilozue, Ikezu, & Ugwu, 2014).

Despite extensive research on avocado seed phytochemicals and solvent extraction efficiency, what remains insufficiently clarified in prior studies is the precise, quantified in vitro inhibitory potency of the methanol extract of avovado seeds.

Most previous investigation have focused broadly on qualitative phytochemical profiling or general in vivo hypoglycemic effect, so the direct enzymatic kinetics and specific inhibitory parameters uncharacterized. Addressing this specific scientific issue is essential to establish a rigorous, biological rationale for avocado seeds as validated AGI agent.

Therefore, this study aims to evaluate and determine the in vitro inhibitory effect of the methanol extract of avocado seeds (*Persia americana Mill)* on alpha glucosidase. The findings from this research are expected to provide accountable scientific evidence regarding the enzymatic mechanism of avocado seeds, ultimately supporting their broader utilization as a standardized herbal candidate from T2DM management .

B. METHODS

1. Tools and Chemicals

Maseration extraction apparatus, ELISA reader (Biotek®), pH meter, microliter pipette (Efendorf®), centrifuge machines, analytical scales (Ohaous®), and vortex. Acarbose as standart from Dexa Medica product, α-glucosidase enzyme derived from *Saccharomyces cerevisiae* recombinant and substrate p-nitrophenyl-α-D-glucopyranoside (PNP) were purchased from Sigma Aldrich, USA.

2. Location, Sample and Population

This research has been completed and conducted at the Chemistry Laboratory of the Faculty of Pharmacy, Muslim Universitas Muslim Indonesia. The sample is the methanol extract of avocado seeds (Persea americana Mill) and the sample population consists of avocado seeds obtained from Mamuju (figure 1), West Sulawesi Province.

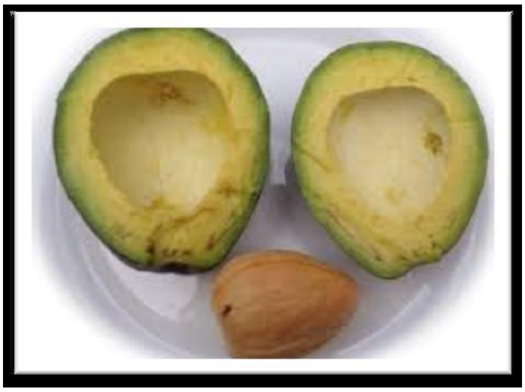

Figure 1. Avocado seed was separated from avocado fruit

3. Extraction Method

The dried sample was powdered and weighed 60.06 grams, then extracted with 150 mL of methanol using the maceration method for 3 x 24 hours, with different methanol used each day. The extract was collected and evaporated to obtain a dry extract, which was then weighed.

4. α-Glucosidase Inhibitory Assay

This assay was done using little modification. The reaction mixture contained 50 µL phosphate buffer pH 7 0.1 M, 25 µL substrate p-Nitrophenyl α-D-glucopyranoside (PNP) 5 mM in phosphate buffer pH 7 0.1 M and 10 µL methanol extract of avocado seeds then added 25 µL α-glucosidase enzyme 1 mg/ml in phosphate buffer pH 7 0.01 M. It was incubated at waterbath for 30 min at 37ºC. After the incubation period is complete, 100 µL Na_2CO_3 200 mM was added (sample solution). Absorbance of the resulting *p*-nitrophenol (*p*NP) was determined at 405 nm by using ELISA reader. Preparing of blank solution, control blank, control sample, standart solution and control standart had to done (Kirana & Ananda, 2020).

5. Data Analysis

Percent Inhibition is calculated by the formula (Kirana & Ananda, 2020) :

$$\% \text{ inhibition} = \frac{B - S}{B} \times 100\ \%$$

Explanation :
B = absorbance of blanks minus absorbance of control blank (C_1-C_0)
S = absorbance of sample minus absorbance of control sample (S_1-S_0)

IC_{50} was calculated using a linear regression equation, the sample concentration as the x-axis and % inhibition as the y-axis. From the equation: y = a + bx can be calculated IC_{50} value by using the formula :

$$IC_{50} = \frac{50 - a}{b}$$

C. RESULT AND DISCUSSION

This research used avocado seed as sample. It cause that phenolics compound of avocado seed more severalfold compared to popular antioxidant sources such as row blueberry (Bangar, et al., 2022). Several studies have shown that phenolic compounds, flavonoids, tannins, and saponins play an important role in providing antioxidant effects (Abidin, Aminah, Razak, & Pratama, 2025). Antioxidant potential is very important for its application in treating conditions related to oxidative stress (Rahayu & Wulandari, 2025). Research results have explained that increased blood sugar levels in diabetes are caused by oxidative stress. Compounds with antioxidant properties can reduce oxidative stress in diabetes sufferers (Maulana, Safitri, & Safira U, 2022). Other research has also concluded that compounds with potent antioxidant effects in treatment are those with antidiabetic potential (Mucha, Skoczynska, Malecka, Hikisz, & Budzisz, 2021).

Avocado seed extract obtained through the maceration method showed a lower IC50 value compared to the reflux extraction method. This indicates that the maceration extract possessed higher antioxidant activity, although both extraction methods demonstrated strong antioxidant activity (Munthe, Riskianto, Juvi, & Novia, 2023).

The phytochemical screening results of avocado seed extract showed that the use of methanol, 70% ethanol, and 96% ethanol solvents produced similar active compound contents, whereas the use of n-hexane solvent resulted in different active compound contents (Azzahra, Sari, & Ashari, 2022).

The methanolic extract of avocado seeds obtained through the maceration method showed phenolic and flavonoid contents of 48.62 mgGA/g and 41.18 mgGA/g, respectively, and demonstrated strong antioxidant activity with an IC50 value of 66.01 ppm (Kurniawan & Rahman, 2022).

Maceration extraction is a simple extraction technique that utilizes solvents with repeated shaking or stirring at room temperature, allowing heat-sensitive compounds to remain stable (Alifah, Faizah, & Swandari, 2023) (Rizqiana & Sudarman, 2023). The selection of an appropriate extraction method for plant samples is an important initial step because it influences the yield of extracts containing the desired chemical compounds. The resulting extract is generally in the form of a crude extract that still contains a complex mixture of various chemical compounds (Stephane, Jules, Batiha, Ali, & Bruno, 2021) (Zhang, Zhao, Dai, & Li, 2023).

The methanol extract of avocado seeds in this study was obtained using the maceration method. The extraction process yielded a dry extract of 8.01 grams from 60.06 grams of dried avocado seed powder. Therefore, the extraction yield is 13.34%.

The use of methanol extract in this research is due to the fact that the methanol extract of avocado seeds contains more phytochemicals than other extracts such as petroleum ether, ethyl acetate, and aqueous. The presence of phytochemicals in the extract can be associated with physiological activities. The methanol extract of avocado seeds contains alkaloids, tannins, flavonoids, triterpenoids, glycosides, and saponins. Alkaloids, one of the phytochemicals, can be used as antimalarials, antidiabetics, antihypertensives, and antitranquilizers (Gangadhara, Valmiki, Radhakrishnan, Geetha, & Ramanathan, 2019) (Ilozue, Ikezu, & Ugwu, 2014).

The potential of methanol extract from avocado seeds (Persea americana Mill) in inhibiting the α-glucosidase enzyme has been tested using p-nitrophenyl-α-D-glucopyranose (PNP) substrate as a model, which represents carbohydrates that will be broken down by the α-glucosidase enzyme into p-nitrophenol and glucose. Acarbose tablets were used as a comparison (positive control).

Acarbose is a natural microbial product obtained from the fermentation process of the microorganism Actinoplanes utahensis. This medication is one of the oral antidiabetic agents from the class of α-glucosidase inhibitors, used for the treatment of T2DM (Ya-jun, et al., 2012). Acarbose acts as an inhibitor of the enzyme glucosidase that breaks down carbohydrates (starch) in the small intestine, thereby delaying glucose absorption in patients with T2DM, particularly postprandial levels (Ke, Jun-Cheng, & Xiao-Ming, 2014). The fundamental principle of this test is the measurement of enzyme activity based on the absorbance of p-nitrophenol, which is the result of the hydrolysis of the PNP substrate. The greater the ability of the plant components to inhibit the enzyme α-glucosidase, the less p-nitrophenol product is formed, resulting in a fading intensity of the visible yellow color (Sri, Siswati, & Efy, 2009). The analysis of α-glucosidase inhibition in this study utilized an enzyme-linked immunosorbent assay (ELISA) reader at a wavelength (λ) of 405 nm.

The inhibition test was conducted on the blank, blank control, test sample (extract), sample control, and acarbose as a positive control/comparison. The extract and acarbose solution were determined based on their ability to inhibit α-glucosidase enzyme activity. The measurements of the blank and blank control aimed to correct the absorption resulting from the potential influence of impure compounds. Similar to the empty control test, the sample control was also performed as a correction factor for the test extract (Lia, Rizki, & Asep, 2015).

The strength of the sample in inhibiting the enzyme is illustrated by the IC50 value. This value represents the concentration that inhibits 50% of enzyme activity. The smaller the IC50 value, the stronger the inhibitory effect. IC50 is calculated using a linear regression equation, as can be seen in figures 2 and 3

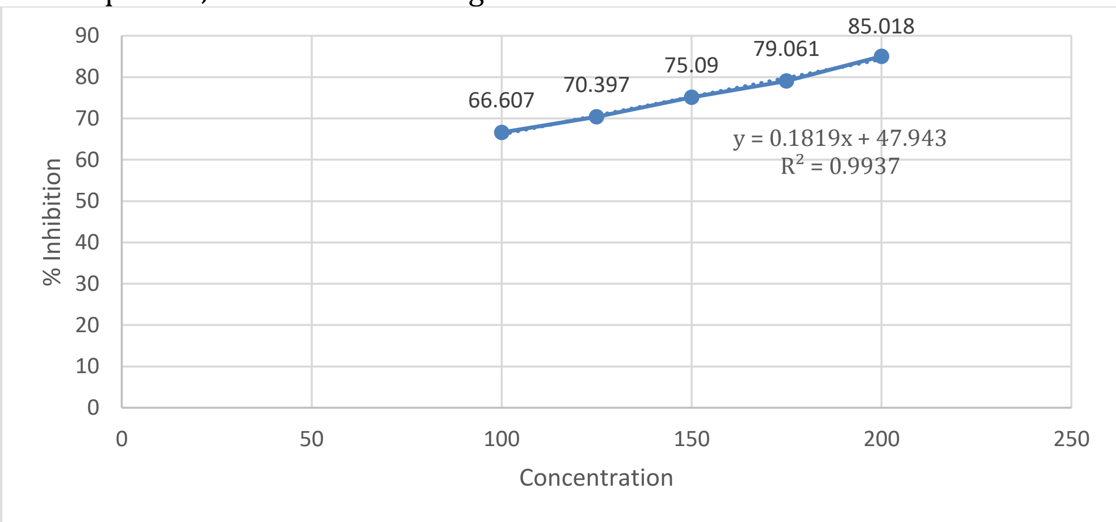

Figure 2. graph of inhibitory activity test of α-glucosidase enzyme by avocado seed extract

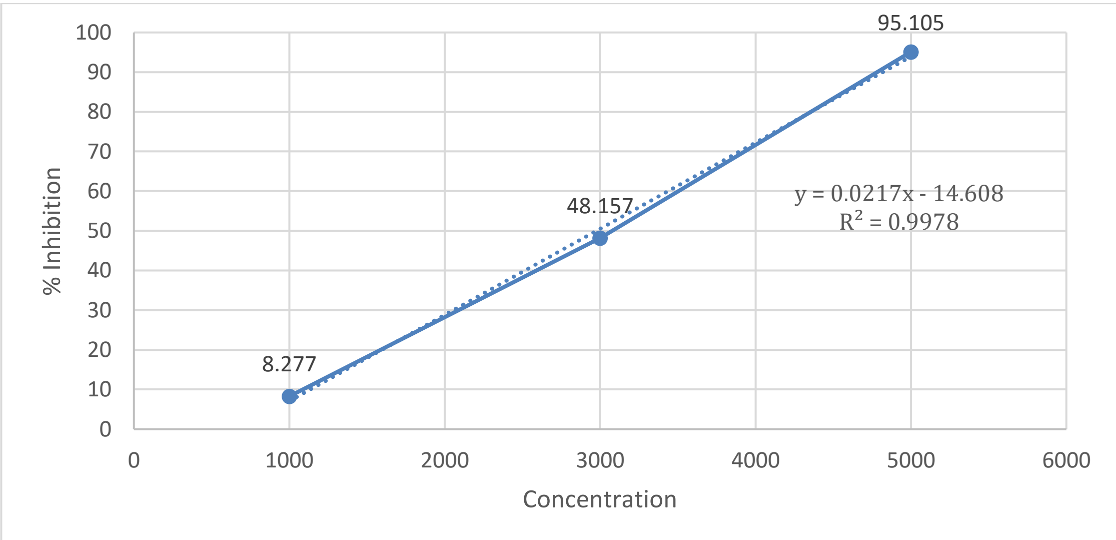

Figure 3. graph of inhibitory activity test of α-glucosidase enzyme by acarbose

The results of the enzyme α-glucosidase inhibition activity test can be seen in Table 1. The IC_{50} value of the methanol extract of avocado seeds is 11.314 μg/mL. In comparison, acarbose has an IC_{50} of 2977.327 μg/mL. Based on Table 1, it can be observed that the methanol extract of avocado seeds achieves an inhibition of 85.018% at a concentration of only 200 μg/mL. Meanwhile, the comparator (acarbose) can achieve an inhibition of 95.105% only when the concentration is increased to 5000 μg/mL. This indicates that the methanol extract of avocado seeds is capable of inhibiting the enzyme α-glucosidase even at low concentrations.

Table 1. The result of inhibitory activity test of α-glucosidase enzyme

Name	Concentration (ppm)	Absorbance	% Inhibition	IC_{50} (ppm)
Avocado seed Extract	100	0.378	66.607	11,314
	125	0.342	70.397	
	150	0.289	75.090	
	175	0.240	79.061	
	200	0.175	85.018	
Acarbose	1000	1.547	8,277	2977,327
	3000	0.884	48,157	
	5000	0.109	95,105	

According to these studies, it can be compared that the methanol extract of avocado seeds is more active as a glucosidase inhibitor than several avocado extracts. The IC50 (mg/mL) of the water extract from avocado leaves, skin, flesh, and seeds are 0.636; 0.080; 0.559, and 0.364, respectively (Adelusi, Oboh, Ayodele, Richard, & Bakare, 2014). The IC50 of the ethanol extract from roasted avocado seeds is 34.300 μg/mL (St, Asriani, & Nurul, 2020). As cited from the other research, the level of inhibition strength against the α-glucosidase enzyme is said to be very active if IC50 ≤25 μg/mL, active if 25 ≤ IC50 ≤50, less active if 50 < IC50 ≤100 μg/mL, and inactive if IC50 > 100 μg/mL (St, Asriani, & Nurul, 2020). Based on that reference, it is known that avocado seed extract methanol is classified as very active in inhibiting the α-glucosidase enzyme, while the comparator acarbose is inactive.

The very high IC50 value may be caused by different enzyme sensitivities to acarbose (Lia, Rizki, & Asep, 2015). Several other acarbose tests in inhibiting the α-glucosidase enzyme from Saccharomyces cerevisiae yeast also showed high IC50 levels (inactive) of 996.02 μg/mL (Ke, et al., 2018), 1431.8 μg/mL (Lia, Rizki, & Asep, 2015), 2450 μg/mL (Kirana & Ananda, 2020), and 8464.0 μg/mL (Malory, Janet, Marlena, & John, 2015).

Based on previous research, it has also been reported that acarbose has low activity on the α-glucosidase enzyme derived from yeast. Acarbose is believed to be more effective in inhibiting the α-glucosidase enzyme derived from mammals, with 100 μg/mL of acarbose able to inhibit up to 34.7% (Jianwei, et al., 2014). Therefore, to this day, acarbose is still commonly used as one of the diabetes medications.

D. CONCLUSION AND SUGGESTIONS

It can be concluded that the methanol extract of avocado seeds (Persea americana Mill) has a very active activity in inhibiting the α-glucosidase enzyme with an IC_{50} of 11.314 μg/mL. For further research, studies can be conducted on the formulation of antidiabetic products.

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