



A-GLUCOSIDASE ENZYME INHIBITOR ACTIVITY OF *ETLINGERA ELATIOR* LEAF EXTRACT WITH SOLVENT VARIATION

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Detail Artikel

Diterima : 25 September 2025
Direvisi : 30 Oktober 2025
Diterbitkan : 31 Oktober 2025

Kata Kunci

*Enzyme inhibition
diabetes mellitus
patikala leaf
α-glucosidase*

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ABSTRACT

Diabetes mellitus is a chronic metabolic disease characterized by hyperglycemia due to impaired insulin production or utilization. The increasing prevalence of diabetes, especially type 2, is a global concern due to the risks of complications it poses. One approach to treating type 2 diabetes is to inhibit the α-glucosidase enzyme to slow down glucose absorption. This research aims to identify the potential of patikala leaf extract as an α-glucosidase enzyme inhibitor, which is not yet specifically available and is still rarely found in scientific literature. The patikala leaves collected are from North Luwu Regency. Extraction using the maceration method with variations in ethanol 70%, ethyl acetate, and n-hexane solvents. The α-glucosidase enzyme inhibition activity was measured using microplates and analyzed to determine the IC₅₀ value. The research results showed that the IC₅₀ 0 values

obtained for 30% ethanol extract were 351.95 $\mu\text{g/mL}$, 70% ethanol extract were 444.83 $\mu\text{g/mL}$, 96% ethanol extract were 362.27 $\mu\text{g/mL}$, ethyl acetate extract were 163.59 $\mu\text{g/mL}$, and n-hexane extract were 294.90 $\mu\text{g/mL}$. In conclusion, based on the research results, the extracts of Patikala leaves with varying solvents exhibited very weak inhibitory activity against the α -glucosidase enzyme, as indicated by an IC_{50} value greater than 100 $\mu\text{g/mL}$.

INTRODUCTION

Diabetes Mellitus (DM) is a group of metabolic disorders characterized by chronic hyperglycemia (increased blood glucose levels above normal limits) resulting from defects in insulin secretion, insulin action, or both. Fundamentally, diabetes occurs because the body is unable to process glucose (sugar) efficiently, which is the body's primary source of energy. This disrupted glucose metabolism process involves several key mechanisms, namely chronic hyperglycemia, insulin resistance, main classifications, pathophysiology, and complications (Leslie dkk., 2023).

Diabetes Mellitus (DM) has been recognized as one of the biggest global public health challenges, with prevalence rates continuing to increase significantly. Data from the International Diabetes Federation (IDF) serves as the primary reference for measuring the burden of this disease worldwide. According to the latest report from the IDF Diabetes Atlas 10th edition (2021), which has more up-to-date data than the 2019 figures, the global burden of diabetes shows an alarming increase. Total Patients (20-79 years): The IDF estimates that at least 537 million people aged 20–79 worldwide had diabetes in 2021. Prevalence Rate: This figure is equivalent to the global prevalence of 10.5% of the total population in the same age group. Future Projections: The IDF projects this number will increase to 643 million by 2030 and reach 783 million by 2045. This increase is driven by demographic changes, increased urbanization, and the adoption of less active lifestyles and unhealthy eating patterns (International Diabetes Federation (IDF), 2021).

The therapeutic approach thru α -glucosidase enzyme inhibition is one of the effective pharmacological strategies for managing diabetes mellitus (DM), particularly for controlling postprandial hyperglycemia (increased blood glucose levels after eating). Mechanism of Action of α -Glucosidase: This enzyme is responsible for catalyzing the hydrolytic cleavage of complex carbohydrates (such as disaccharides and oligosaccharides) into monosaccharides (primarily glucose) (Khan dkk., 2024).

There is one therapeutic approach that can be used to treat diabetes mellitus, which is by inhibiting enzymes related to glucose absorption in the body, such as the enzyme α -glucosidase. The enzyme α -glucosidase functions to accelerate the absorption of glucose by the small intestine by catalyzing the hydrolytic cleavage of oligosaccharides into monosaccharides, which causes an increase in blood glucose levels in the body after eating. To slow or delay the absorption of glucose in the intestine, which can prevent postprandial blood glucose levels from rising, α -glucosidase enzyme inhibitors are needed (Fatmawati dkk., 2021).

Patikala plant (*Etlingera elatior* (Jack) RM. Smith) belongs to the Zingiberaceae family and originates from Indonesia. Then, this plant has spread widely throughout the regions of Indonesia and is known by various names, including "kencong" or "kincung" in North Sumatra, "kecombrang" in Java, "honje" in Sunda, "bongkot" in Bali, "sambuang" in

West Sumatra, "bunga katan" in Malaysia, and "patikala" in South Sulawesi. Some previous studies have shown that the leaves of *Andrographis paniculata* have various pharmacological activities, including antioxidant, antimicrobial, and anti-inflammatory effects (Utami dkk., 2024).

Patikala leaves contain various bioactive compounds, including flavonoids, tannins, and terpenoids, which potentially have α -glucosidase enzyme inhibitory activity. Previous studies have shown that extracts from other parts of this plant, such as the flowers and rhizomes, have antidiabetic activity (Utami, Yulianty, dkk., 2023).

Based on the background above, the results of this research are expected to provide scientific information regarding the potential of patikala leaves as a natural antidiabetic agent, specifically thru the mechanism of α -glucosidase enzyme inhibition. Additionally, this research can also serve as a foundation for the further development of herbal products for diabetes management based on local Indonesian plants.

Method

Research Type

The research conducted was laboratory-scale experimental research. This research was carried out at the Pharmaceutical Biology Laboratory and the Phytochemistry Laboratory of the Integrated Research Laboratory, the Research Laboratory of the Faculty of Health Sciences, Almarisah Madani University.

Tools and Materials

The tools that will be used in this research include a stirring rod, porcelain crucible, funnel, beaker, chemical glass, measuring cylinder, tweezers, microliter pipette (Eppendorf), dropper pipette, volumetric pipette, horn spoon, analytical balance, vial, water bath (B-One), 96-well plate, and Elisa Reader (Epoch). The materials used in this study include distilled water, n-hexane, ethyl acetate, ethanol at concentrations of 96%, 70%, and 30%, Acarbose, α -glucosidase enzyme, 36 μ L phosphate buffer solution with a pH of 6.8, sodium carbonate solution (Na_2CO_3), p-nitrophenyl-D-glucopyranoside (pNPG) substrate, DMSO, and patikala leaves (Chaudhary, P. dkk., 2024).

Sample Preparation

The collected patikala leaves are separated from dirt, then washed with running water to remove any adhering impurities. After that, the leaves are drained and chopped. The drying process is done by air-drying until the leaves crumble when squeezed, which indicates that drying is complete. After drying, the samples are sorted to remove damaged or interfering materials (Utami, Yulianty, dkk., 2023).

Sample Extraction

The maceration extraction method was performed using various solvents, namely 30% ethanol, 70% ethanol, 96% ethanol, ethyl acetate, and n-hexane. Weigh 20 grams of patikala leaf (*Etlingera elatior* (Jack) R.M. Smith) simple powder that has been sieved thru a 16-mesh screen and macerate it with 200 mL of each solvent variation, slowly stirring until the solvent completely covers the patikala leaf powder. Maceration was carried out for 72 hours, with the first 8 hours involving stirring. The mixture was then filtered using filter paper, and the resulting liquid extract (macerate) was evaporated using a water bath at a temperature of

50°C. The concentrated extract was then weighed to determine the yield percentage, which was calculated using the following formula (Utami, Imrawati, dkk., 2023):

$$\% \text{ yield} = \frac{\text{Extract Weight}}{\text{Sample Weight}} \times 100\%$$

Phytochemical screening using Thin Layer Chromatography (TLC)

Screening was performed using silica gel F254 plates. TLC plates were prepared with dimensions of 1 cm x 7 cm using a pencil (1 cm from the bottom of the plate and 0.5 cm from the top). The silica gel F254 TLC plates were first activated by heating in an oven at 110°C for 30 minutes to remove any moisture adsorbed in the plate. The eluent mixture is placed in the chamber, which is then tightly closed, and the saturation process is carried out using filter paper as a reference. This saturation is done to equalize the vapor pressure of the eluent so that separation can proceed well (Imrawati dkk. 2023).

Ethanol extract of tekelan leaves from various drying techniques was taken at 25 mg and dissolved in ethanol. Then the extract was spotted 1 cm from the bottom edge of the plate using a capillary tube. The extract spotted on the TLC plate is then eluted with the eluent. The TLC plate is placed in a chamber containing saturated eluent, and the chamber is then tightly closed until the eluent reaches the top line. Then the plate is lifted, dried, and sprayed with the reagent. The appearance of the spots can be seen under a 366 nm UV lamp (Imrawati dkk. 2023).

This test was conducted on several compounds, including (Mus, Suwahyuni dkk. 2023) :

1. Alkaloid

The TLC plate, spotted with the diluted extract using a mobile phase of n-hexane:ethyl acetate (8:2), was then observed using Dragendorff's reagent. A positive result is indicated by the appearance of brown to yellow spots.

2. Flavonoid

The TLC plate, spotted with the diluted extract using a mobile phase of n-hexane:ethyl acetate (8:2), was then observed with a spot developer using 1% AlCl_3 reagent. Positive results were indicated by the appearance of blue fluorescent spots when observed under 366 nm UV light.

3. Tannin

Mobile phase: n-Hexane:ethyl acetate (8:2), with spot visualization using 5% FeCl_3 reagent. A positive reaction is indicated by the formation of a black spot.

4. Steroid

The mobile phase used was n-hexane:ethyl acetate in a ratio of 8:2 mL, and spot visualization was performed using the Liebermann-Burchard spray reagent. A positive result for steroid compound content is indicated by the formation of green or blue spots, while dark blue or blackish-green spots also indicate the presence of steroids.

Inhibitor α -Glukosidase Activity

Preparation of Phosphate Buffer Solution (pH 6.8)

Disodium phosphate (Na_2HPO_4) of 1.778 grams and 1.560 grams of monobasic sodium phosphate (NaH_2PO_4) were each dissolved in a 100 mL volumetric flask with distilled

water and brought up to the flask's mark. The mixture of the two solutions was then measured using a pH meter until it showed a pH of 6.8.

α -Glucosidase Solution

One mg of α -glucosidase enzyme was dissolved in 1 mL of pH 6.8 phosphate buffer, resulting in an α -glucosidase enzyme solution (0.025 U/mL). A concentration of 0.15 U/mL was then prepared. The α -glucosidase enzyme solution was made by pipetting 15 μ L and then bringing the volume up to 1 mL using pH 6.8 phosphate buffer.

4-p-nitrophenyl- α -D-glucopyranoside (pNPG) substrate solution

The p-nitrophenyl α -D-glucopyranoside (pNPG) substrate solution was prepared by weighing 6.025 mg of pNPG and dissolving it in phosphate buffer (6.8) to a final volume of 5 mL, resulting in a concentration of 2 mM.

Sodium Carbonate (Na_2CO_3) Solution

Sodium carbonate (Na_2CO_3) 200 mM solution was prepared by weighing 2.12 grams of sodium carbonate powder and dissolving it in distilled water to a final volume of 100 mL.

Preparation of Acarbose Solution

Preparation of the acarbose 1000 μ g/mL stock solution was done by weighing 2 mg of acarbose and dissolving it in 1 mL of pH 6.8 phosphate buffer. Then, a series of concentrations were prepared: 100 μ g/mL; 50 μ g/mL; 25 μ g/mL; 12.5 μ g/mL; and 6.25 μ g/mL. This was achieved by taking 100 μ g/mL from the 1000 μ g/mL solution and adding pH 6.8 phosphate buffer to make a final volume of 100 μ g/mL. From the 100 μ g/mL solution, 500 μ L was taken and made up to 500 μ L with pH 6.8 phosphate buffer, resulting in a 50 μ g/mL solution. This process was continued until the final concentration was reached.

Preparation of Sample Solution

A sample stock solution of 10,000 μ g/mL was prepared. For each sample, 30% ethanol, 70% ethanol, 96% ethanol, ethyl acetate, and n-hexane extracts were used. Weigh out 10 mg of the sample, dissolve it in 100 μ L of Dimethyl Sulfoxide (DMSO), and then add 900 μ L of phosphate buffer at pH 6.8.

Determination of Maximum Wavelength

A 50 μ L sample solution and 20 μ L phosphate buffer (pH 6.8) were mixed, followed by the addition of 15 μ L pNPG substrate and 15 μ L α -glucosidase enzyme solution. The mixture was then incubated for 15 minutes at 37°C. The volume was then brought up to 100 μ L with sodium carbonate, and the absorbance was measured at a wavelength of 400 nm using a microplate reader.

Inhibitor α -Glukosidase Activity Test

Blank Test

The reagent mixture used in this test consists of 60 μ L phosphate buffer (pH 6.8), plus 20 μ L 2 mM pNPG, and 20 μ L α -glucosidase solution (0.025 U/mL). The mixture was incubated for 15 minutes at 37°C. The reaction was stopped by adding 100 μ L of 200 mM sodium carbonate solution. The assay was performed in 3 replicates, and the sample absorbance was measured using a microplate reader at a wavelength of 400 nm, also with 3 replicates.

Acarbose Comparison Test

This mixture consisted of 30 μ L of acarbose solution, 40 μ L of phosphate buffer solution (pH 6.8), and 17 μ L of pNPG substrate, which were added to a 96-well plate. The mixture was incubated for 5 minutes at 37°C, then 17 μ L of 0.5 U/mL α -glucosidase enzyme solution was added and incubated again for 15 minutes at 37°C. After incubation, the volume of the mixture was adjusted with sodium carbonate to reach 100 μ L. Testing was conducted with concentration variations of 100 μ g/mL; 50 μ g/mL; 25 μ g/mL; 12.5 μ g/mL; 6.25 μ g/mL; and 3.125 μ g/mL. Absorbance was measured at a wavelength of 400 nm with 3 replicates.

Data Analysis

The technique for analyzing data involves calculating the concentration of the sample solution as an inhibitor in inhibiting 50% of the α -glucosidase enzyme activity (IC₅₀). The IC₅₀ value is obtained by creating a curve showing the relationship between concentration and the percentage of inhibition by the sample against α -glucosidase enzyme activity.

The percentage of extract's inhibitory activity against α -glucosidase was calculated using the formula:

$$\% \text{ inhibition} = \frac{\text{Abs Blank} - \text{Abs Sample}}{\text{Absorb Blank}} \times 100\%$$

(Mechchate dkk., 2021)

The calculation results will be entered into a linear equation.

The resulting linear equation is used to obtain the IC₅₀ value. The IC₅₀ value is the concentration obtained when the percentage of inhibition is 50 from the equation $y = ax + b$. When the percentage of inhibition is 50, the formula for calculating the IC₅₀ value becomes : $x = \frac{50 - b}{a}$. (Mechchate dkk., 2021)

RESULT AND DISCUSSION

The inhibition testing of patikala leaf extract (*Etlingera elatior* (Jack) R.M Smith) against the α -glucosidase enzyme began with sample collection from the growth location, namely Mariri Hamlet, Banyuurip Village, Bone-bone District, North Luwu Regency. In this study, the crude drug was obtained from the residue of a previous study that had been dried for 3 days. Drying aims to reduce the water content so that the crude drugs are not easily damaged and do not grow mold, and to maximize the extraction process. The patikala leaf simplisia is then pulverized into a smaller form to increase the surface area of the patikala leaf simplisia in contact with the solvent, thereby facilitating the extraction process. Method Selection The selection of the extraction method is based on the characteristics of flavonoid secondary metabolites, which are secondary metabolites that are not resistant to high temperatures. Extraction must be carefully considered to obtain maximum extraction results. and can be oxidized by high temperatures. Therefore, the maceration method is suitable for obtaining better flavonoid secondary metabolites (Murniyati dkk., 2021).

This study used 3 extraction solvents. The process of making an extract, the extracting liquid is the solvent used to draw out or separate the active compounds from the material and from other contained compounds. Research using three types of extraction solvents (70%

ethanol, ethyl acetate, and n-hexane) to create plant extracts is a standard approach in phytochemistry and pharmacognosy. This method is known as fractional extraction and aims to separate chemical compounds based on their polarity levels (Dirir dkk., 2022).

The spraying liquid or solvent works on the principle of "like dissolves like" (similar compounds dissolve in similar solvents). The use of three solvents with different polarities allows for the extraction of different groups of compounds. Bioactive compounds responsible for alpha-glucosidase inhibition, such as flavonoids and polyphenols, generally have moderate to high polarity. Therefore, the extracts that most frequently show the strongest inhibitory activity are ethanol extracts or ethyl acetate extracts (Ulfa, D. A., et al., 2024).

The selection of the extraction solvent is the most crucial step in the process of extracting bioactive compounds from natural materials. This decision directly affects the chemical composition, pharmacological activity, and economic value of the resulting extracts. The main factors in selecting a solvent are selectivity, ease of use, the process involved, cost-effectiveness, environmental friendliness, and safety. The yield of an extract can be influenced by several factors, one of which is the type of solvent and its concentration. Yield is the value obtained from the ratio of the dry weight of the product produced to the weight of the raw material (Cannavacciuolo dkk., 2024). Hasil ekstraksi dapat dilihat pada tabel 1.

Tabel 1. Yield of Patikala Leaf Extract

Sample	Simplicia Weight (g)	Solvent	Extract Weight (g)	Randemen (%)
Daun Patikala	20	Ethanol 70%	4,492	22,46
		Ethyl asetat	0,931	4,65
		n-Hexan	0,347	1,73

Based on the research results in Table 1. The average yield value for each extraction solvent was highest with ethanol, as ethanol can dissolve almost all compounds present in the sample, both polar and non-polar. Additionally, ethanol can degrade cell walls, making it easier for bioactive compounds to be released from plant cells. Ethanol (absolute ethanol or in a mixture with water) is widely recognized in the field of phytochemistry as a highly efficient solvent, often yielding the highest total extract yields compared to other single solvents such as ethyl acetate, chloroform, or n-hexane. This advantage is based on two main mechanisms: its broad solvent power and its ability to facilitate the release of compounds (Dutta, S., et al., 2024).

Tabel 2. Phytochemical Screening Results of Patikala Leaf Extract Using TLC Method

Compound class	Reagen	Extract	Result	Ket .
Alkaloid	Dragendorf	Ethanol 70%	Brownish-orange stain	(+)
		Ethyl asetat	Brownish-orange stain	(+)
		n-Hexan	Brownish-orange	(+)

		stain		
Flavonoid	AlCl ₃ 1%	Ethanol 70%	Blue fluorescent spots	(+)
		Ethyl acetate	Blue fluorescent spots	(+)
		n-Hexan	Blue fluorescent spots	(+)
Tannin	FeCl ₃	Ethanol 70%	Black-colored stain	(+)
		Ethyl acetate	Black-colored stain	(+)
		n-Hexan	Black-colored stain	(+)
Steroid	Lieberman Buchard	Ethanol 70%	No spots/stains	(-)
		Ethyl acetate	Green-blue colored stain	(+)
		n-Hexan	Green-blue colored stain	(+)
Terpenoid	Lieberman Buchard	Ethanol 70%	No spots/stains	(-)
		Ethyl acetate	Dark blue or blackish green spots	(+)
		n-Hexan	Dark blue or blackish green spots	(+)

Based on the results obtained from phytochemical screening using the TLC method, it can be seen that the variation of solvent for the patikala leaf extract using n-hexane:ethyl acetate (8:2) contains positive compounds of alkaloids, flavonoids, tannins, steroids, and terpenoids. This is in accordance with research conducted by (Utami dkk., 2024).

In this α -glucosidase enzyme activity test, variations in the concentration of 70% ethanol, ethyl acetate, and n-hexane extracts from patikala leaves were used, specifically 2,000 ppm, 1,000 ppm, 500 ppm, 250 ppm, 125 ppm, and 62.5 ppm. This α -glucosidase enzyme inhibition activity test was conducted to determine the activity of compounds contained in the extract that can be used as inhibitors. The percentage inhibition value of the sample will be inversely proportional to the absorbance value of p-nitrophenol, which will decrease.

In this test, a phosphate buffer at pH 6.8 was used and incubated at 37°C. These pH and temperature conditions are consistent with those described by (Aldrich, S., 2022), pH and temperature greatly affect enzyme activity, so the pH and temperature of the environment need to be considered to ensure the enzyme's activity reaches optimal conditions. The temperature commonly used in various literature and in Sigma's product information data is 37°C. However, there are also literature sources that use a temperature range of 30-45°C. From the research data from Alrashidi dkk., (2022), The temperature variation tests were 30,

40, and 45°C. The results obtained showed an increase in α -glucosidase activity from 30°C to 37°C, and a significant decrease in α -glucosidase activity at 40°C. After obtaining the absorbance values from the extract, sodium carbonate (Na_2CO_3) was added to the solution to stop the enzymatic reaction.

Enzyme activity is measured based on the absorbance of the yellow color of p-nitrophenol, which is a product of the reaction between the enzyme α -glucosidase and the substrate p-nitrophenyl-D-glucopyranoside, resulting in D-glucose and p-nitrophenol. The presence of inhibitors in the compounds contained in 30% ethanol, 70% ethanol, 96% ethanol, ethyl acetate, and n-hexane extracts from Patikala leaves is expected to inhibit the substrate from binding to the active site of the enzyme, thus reducing the intensity of the yellow color produced (Etsassala dkk., 2022).

For the measurement, an Enzyme-Linked Immunosorbent Assay (ELISA reader) instrument was used, operated at a wavelength of 405 nm. The ELISA reader has several advantages, including relatively fast processing techniques, high sensitivity, and the ability to produce more accurate data even with a very small amount of test solution used (Dirir dkk., 2022)

According to research cited from Simarmata, D. F., et al., (2023) the level of inhibitory strength against the α -glucosidase enzyme is categorized as very strong if the IC_{50} value is $\leq 25 \mu\text{g/mL}$, strong if the IC_{50} is in the range of 25-50 $\mu\text{g/mL}$, weak if the IC_{50} is between 50-100 $\mu\text{g/mL}$, and very weak if the IC_{50} is $> 100 \mu\text{g/mL}$. The results of the inhibition tests for 70% ethanol, ethyl acetate, and n-hexane extracts from patikala leaves are shown in the following table.

Table 3. Inhibition Results Against α -glucosidase Enzyme of Patikala Leaf Extract

Extract	Concentration ($\mu\text{g/mL}$)	Absorbance	% Average inhibition	IC_{50} Value ($\mu\text{g/mL}$)	Category
Ethanol extract 30%	15.625	0.610			
		0.58			
		0.597			
		0.570			very weak
	31.25	0.567	27.152		
		0.572			
		0.533			
	62.5	0.537	31.67	351.951	
		0.533			
		0.471			
Ethanol extract 70%	125	0.531	37.382		
		0.467			
		0.447			
		0.483	41.048		
	250	0.453			
		0.624			very weak
		0.580	17.86		
	31.25	0.723			
		0.574			
		0.59	24.04	444.831	
		0.618			
	62.5	0.555			
		0.546	24.936		

		0.66		
		0.591		
	125	0.494	28.346	
		0.596		
		0.449		
	250	0.496	36.317	
		0.549		
		0.635		
	15.625	0.628	17.945	
		0.662		
		0.595		very weak
	31.25	0.651	24.552	
		0.524		
		0.567		
Ethanol extract	96%	62.5	26.768	362.267
		0.572		
		0.579		
		0.553		
	125	0.571	28.9	
		0.544		
		0.467		
	250	0.485	40.622	
		0.441		
		0.594		
	15.625	0.559	22.506	
		0.665		
		0.507		very weak
	31.25	0.533	32.438	
		0.545		
		0.544		
n-hexan extract		62.5	37.936	294.903
		0.492		
		0.420		
		0.463		
	125	0.464	40.92	
		0.459		
		0.454		
	250	0.428	44.117	
		0.429		
		0.616		
	15.625	0.648	22.208	
		0.561		
		0.519		
	31.25	0.485	33.248	very weak
		0.562		
		0.482		
etil asetat extract		62.5	40.878	163.592
		0.468		
		0.437		
		0.414		
	125	0.434	46.248	
		0.413		
		0.314		
	250	0.297	60.272	
		0.321		
	Blank	0.782		

From the data on % inhibition, the IC_{50} values obtained for 30% ethanol extract of patikala leaves were (351.95 μ g/mL), 70% ethanol extract (444.83 μ g/mL), 96% ethanol extract (362.27 μ g/mL), ethyl acetate extract of patikala leaves was 163.59 μ g/mL, and n-hexane extract of patikala leaves was 294.90 μ g/mL, indicating that patikala leaf extracts with variations in solvent or extraction method exhibited very weak α -glucosidase enzyme inhibition activity.

Table 4. Results of Comparing Positive Control Inhibition (Acarbose)

Concentration (μ g/mL)	Replication	Absorbance	% inhibition average	IC_{50} Value (μ g/mL)	Category
0.468	1	0.780			
	2	0.792	-1.331		
	3	0.787			
0.9375	1	0.745			
	2	0.747	5.068		
	3	0.718			
1.875	1	0.677			
	2	0.688	11.469	83.21	Weak
	3	0.696			
37.5	1	0.60			
	2	0.578	24.183		
	3	0.587			
7.5	1	0.48			
	2	0.48	37.242		
	3	0.501			
Blanko		0.776			

In the testing of α -glucosidase enzyme inhibition activity, acarbose was used as a comparison. Acarbose is used because it is an easily obtainable α -glucosidase inhibitor and is commonly used as a test standard for α -glucosidase enzyme inhibition. The results from the data on the percentage of α -glucosidase enzyme inhibition in Table 4 show that the IC_{50} value for the positive control acarbose is 83.21 μ g/mL, indicating that the positive control acarbose exhibits weak α -glucosidase enzyme inhibition activity. The IC_{50} values obtained for each sample were compared to the IC_{50} value of acarbose to assess the comparison of α -glucosidase enzyme inhibition.

Based on research conducted by Saeedi, M. A. dkk., (2022), this study demonstrates that after the crude extract is fractionated (separated), the purified fractions (such as the ethyl acetate fraction) exhibit significantly stronger alpha-glucosidase inhibitory activity compared to the original extract. This proves that the crude extract contains "impurities" that weaken its activity. It is known that crude extracts often yield less active results due to the mixture of compounds. It falls into the very weak category in inhibiting α -glucosidase enzyme activity. This is suspected because the extract is still a crude extract, where the compounds it contains

are still a mixture, so some compounds may not have activity as α -glucosidase enzyme inhibitors.

Alpha-glucosidase inhibitory activity is directly proportional to the purity of the phenolic compound group. The unpurified crude extract has low activity because the phenolic concentration is masked by other plant matrix components. In the study Nguyen, T. H., dkk., (2023), it was found that non-selective extraction techniques (yielding crude extracts) provided moderate/weak enzyme inhibition results. Conversely, techniques that target the enrichment of bioactive compounds significantly lower the IC_{50} value.

This is also in line with research Sulastri, E., Zubaydah, W. O. S. & Sahidin, I., (2023) which shows that stepwise fractionation increases antidiabetic potential. Purer fractions have better activity than crude ethanol extracts due to the loss of interfering compounds. It explicitly discusses that the complexity of metabolites in crude extracts often leads to "false negative" results or weak activity, and the true activity is only apparent after partial purification

Conclusion

Based on the research results obtained, extracts with variations in solvents or extraction methods were analyzed. The extract of Patikala leaves showed inhibitory activity against the α -glucosidase enzyme, but this activity was categorized as very weak because its IC_{50} value was $> 100 \mu\text{g/mL}$. In contrast, the control (acarbose) was categorized as weak because its IC_{50} value was $50-100 \mu\text{g/mL}$.

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