

Evaluation of α -Glucosidase Inhibitor Activity of Ethanol Extract of Soursop Leaf (*Annona muricata* L.)

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Abstract

Soursop (*Annona muricata* L.) is a plant widely used in traditional medicine, where people typically consume soursop leaf infusions or supplements to help regulate blood sugar levels. This plant contains several bioactive compounds, including flavonoids, quercetin, tannins, alkaloids, fatty acids, phytosterols, morinol alcohol, and annonol. Among these, flavonoids, quercetin, and tannins are suspected to have antidiabetic properties. In recent years, interest has increased in the potential of natural products, especially plant extracts, as alternative therapeutic agents for managing diabetes, particularly through the inhibition of α -glucosidase, an enzyme involved in carbohydrate digestion and glucose absorption.

Despite the widespread use of soursop, there is limited scientific evidence evaluating its α -glucosidase inhibitory activity. This research aims to assess the potential of ethanol extract of soursop leaves (*Annona muricata* L.) as an α -glucosidase inhibitor using a microplate reader. In this study, the inhibition of α -glucosidase was determined by the enzymatic reaction between α -glucosidase and the substrate p-nitrophenyl- α -D-glucopyranoside (PNPG), measured spectrophotometrically using a microplate reader.

The results of the study indicate that the ethanol extract of soursop leaves exhibited moderate inhibition of α -glucosidase, with an IC₅₀ value of 764.14 μ g/mL. While the extract demonstrated some inhibitory potential, it was less potent compared to standard α -glucosidase inhibitors. These findings suggest that although the ethanol extract of soursop leaves has α -glucosidase inhibitory activity, it may require further optimization or combination with other compounds to enhance its effectiveness. This research contributes to the growing body of evidence regarding the antidiabetic properties of soursop and provides insights into its potential as a functional food or herbal supplement for managing diabetes.

Keywords: Soursop (*Annona muricata* L.), α -Glucosidase inhibitor, Ethanol extract, Antidiabetic activity, Microplate reader

1. Introduction

Diabetes mellitus has emerged as a global epidemic, posing significant challenges to public health and leading to considerable economic burdens, particularly in developing countries such as Indonesia (Yunita et al., 2022). Diabetes is a chronic, non-communicable metabolic disorder characterized by impaired insulin action, secretion, or both, which results in disturbances in carbohydrate, protein, and fat metabolism. This disease is a leading cause of blindness, stroke, kidney failure, and limb amputations, making it a critical health concern worldwide (Aynalem & Zeleke, 2018).

One of the key enzymes involved in carbohydrate metabolism is α -glucosidase, which is primarily found on the surface of small intestinal cells. This enzyme catalyzes the breakdown of complex

oligosaccharides into monosaccharides, such as glucose, which are then absorbed into the bloodstream. Inhibiting α -glucosidase activity has become a therapeutic approach for managing Type 2 Diabetes Mellitus (T2DM) by delaying glucose absorption, thereby reducing postprandial hyperglycemia (Kusumawati et al., 2021). Alpha-glucosidase inhibitors (AGIs) are a class of oral medications commonly used to treat T2DM. These inhibitors competitively block the enzyme, preventing the breakdown of complex carbohydrates into simple sugars that can be absorbed by the body (Akmal & Wadhwa, 2022). Currently, three AGIs are in clinical use: acarbose, miglitol, and voglibose, all of which have demonstrated efficacy in managing blood glucose levels and reducing cardiovascular risks in individuals with impaired glucose tolerance (Hanefeld & Mertes, 2019).

In addition to pharmaceutical interventions, traditional medicine has long utilized natural remedies for managing diabetes, and soursop (*Annona muricata* L.) is one such plant. Soursop leaves are commonly used in folk medicine in various regions, including Indonesia, where they are consumed as a tea or in supplement form to help regulate blood glucose levels (Ayuningtiyas et al., 2022). Soursop, native to the Caribbean, South America, and Central America, is now widely cultivated across Indonesia. The plant contains numerous bioactive compounds, including flavonoids, tannins, alkaloids, fatty acids, phytosterols, morinol alcohol, and annonol. Among these compounds, flavonoids, quercetin, and tannins have been identified as potentially contributing to the plant's antidiabetic properties. Flavonoids act as antioxidants and may help regenerate damaged β -pancreatic cells, thereby addressing insulin deficiency. Tannins, on the other hand, inhibit glucose absorption in the small intestine, delaying glucose uptake after meals and mitigating postprandial hyperglycemia. Moreover, flavonoids, especially quercetin, are believed to inhibit glucose transporter 2 (GLUT2) in the intestinal mucosa, reducing glucose absorption (Ajie, 2015). It has been shown that flavonoids with catechol groups in rings A or B and a hydroxyl group at C3 exhibit the strongest α -glucosidase inhibitory activity, whereas modifications such as the absence of a conjugated double bond between C2 and C3 or the presence of a ketone group at C4 reduce this activity significantly (He et al., 2019).

Despite the growing interest in soursop's potential antidiabetic effects, there is a lack of scientific evidence specifically evaluating the α -glucosidase inhibitory activity of soursop leaf extracts. Therefore, the aim of this study is to evaluate the inhibitory activity of ethanol extract from soursop leaves (*Annona muricata* L.) on α -glucosidase using a microplate reader. By examining this activity, we hope to contribute to a better understanding of soursop's potential as a natural remedy for managing diabetes.

2. Materials and Methods

Equipment

The following equipment was used in the research: microplate reader, 96-well microplate, micropipettes, dropper pipettes, analytical balance, oven, drying oven for simplicia, microcentrifuge tubes, vortex mixer, and vials.

Materials

The materials used in this study include: soursop leaves (*Annona muricata* L.) obtained from the North Toraja District, South Sulawesi, 70% ethanol, α -glucosidase enzyme, acarbose, phosphate buffer (pH 7.0), p-nitrophenyl- α -D-glucopyranoside (PNPG), and sodium carbonate (Na_2CO_3).

Sample Collection

The soursop leaves (*Annona muricata* L.) were collected from Tagari, Tallunglipu District, North Toraja Regency, South Sulawesi Province.

Sample Preparation

The collected soursop leaves were cleaned to remove dirt and debris, then washed thoroughly with running water. After washing, the leaves were drained and dried in a drying oven at 40°C for 3 days. Once dried, the leaves were sorted, ground into powder using a mesh 16 sieve, and prepared for extraction.

Extraction Process

A total of 200 grams of soursop leaf powder was weighed and placed into a maceration container. To this, 1,500 mL of 70% ethanol was added, maintaining a ratio of 1:7.5. The mixture was left to macerate for 3 days in a closed container, protected from direct sunlight, with occasional stirring every 24 hours. After the maceration period, the mixture was filtered, and the remaining residue was subjected to a second round of maceration with fresh 70% ethanol. The remaceration was repeated twice, each time for 24 hours. The resulting extracts were collected and concentrated by evaporation to obtain a thick ethanol extract.

α -Glucosidase Inhibition Activity Assay

Preparation of Ethanol Extract Solution

The stock solution of the ethanol extract was prepared at a concentration of 10,000 ppm. To prepare this, 10 mg of the ethanol extract was weighed and dissolved in 1 mL of phosphate buffer (pH 7.0) in a microcentrifuge tube. A series of concentrations were then prepared by sequential dilution, starting from 1000 ppm, 500 ppm, 250 ppm, 125 ppm, 62.5 ppm, 31.25 ppm, and 15.625 ppm, by taking 100 μ L of the 10,000 ppm stock solution and adding 900 μ L of phosphate buffer (pH 7.0), yielding the 1000 ppm solution. The dilution was continued in the same manner until the final concentrations were achieved.

Preparation of Acarbose Solution

Acarbose stock solution (1000 ppm) was prepared by weighing 10 mg of acarbose and dissolving it in 10 mL of phosphate buffer (pH 7.0). A series of concentrations were then prepared by diluting the stock solution to obtain concentrations of 100 ppm, 50 ppm, 25 ppm, 12.5 ppm, and 6.25 ppm. The dilution procedure was the same as described for the ethanol extract solution.

Preparation of α -Glucosidase Solution (0.025 U/mL)

α -Glucosidase enzyme (1 mg) was weighed and dissolved in 1 mL of phosphate buffer (pH 7.0) to obtain a 10 U/mL enzyme solution. A working solution of α -glucosidase (0.025 U/mL) was prepared by pipetting 25 μ L of the 10 U/mL enzyme solution and diluting it with 10 mL of phosphate buffer (pH 7.0).

Preparation of PNPG Substrate (1.99 mM)

The PNPG substrate was prepared by weighing 3.000 mg of p-nitrophenyl- α -D-glucopyranoside (PNPG) and dissolving it in 5 mL of phosphate buffer (pH 7.0) to achieve a 1.99 mM concentration.

Blank Control Assay

For the blank control, each reaction mixture consisted of 50 μ L of phosphate buffer (pH 7.0), 50 μ L of 2 mM PNPG, and 20 μ L of the α -glucosidase solution (0.025 U/mL). The mixture was incubated at

37°C for 30 minutes, and the reaction was stopped by adding 80 µL of 0.2 M sodium carbonate solution. Absorbance was measured using a microplate reader at a wavelength of 400 nm, with three replicates.

Acarbose Control Assay

For the acarbose control, 50 µL of each prepared acarbose concentration (6.25 ppm, 12.5 ppm, 25 ppm, 50 ppm, and 100 ppm) was added to 50 µL of 2 mM PNPG and 20 µL of α-glucosidase solution (0.025 U/mL). The mixture was incubated at 37°C for 30 minutes, and the reaction was stopped by adding 80 µL of 0.2 M sodium carbonate solution. Absorbance was measured using a microplate reader at 400 nm, with three replicates for each concentration.

Ethanol Extract Sample Assay

For the ethanol extract sample assay, 50 µL of each prepared extract concentration (1000 ppm, 500 ppm, 250 ppm, 125 ppm, 62.5 ppm, 31.25 ppm, and 15.625 ppm) was added to 50 µL of 2 mM PNPG and 20 µL of α-glucosidase solution (0.025 U/mL). The mixture was incubated at 37°C for 30 minutes, and the reaction was stopped by adding 80 µL of 0.2 M sodium carbonate solution. Absorbance was measured using a microplate reader at 400 nm, with three replicates for each concentration.

Color Control Assay for Ethanol Extract Samples

For the color control assay, 50 µL of each prepared ethanol extract concentration (1000 ppm, 500 ppm, 250 ppm, 125 ppm, 62.5 ppm, 31.25 ppm, and 15.625 ppm) was added to 70 µL of 2 mM PNPG and incubated at 37°C for 30 minutes. The reaction was stopped by adding 80 µL of 0.2 M sodium carbonate solution, and the absorbance was measured using a microplate reader at 400 nm.

Data Analysis

The IC₅₀ value (the inhibitor concentration required to inhibit 50% of enzyme activity) was determined by plotting the percentage of inhibition against the concentration of the extract and deriving the regression equation.

3. Result and Discussion

This study investigated the α-glucosidase inhibitory activity of ethanol extract from soursop leaves (*Annona muricata* L.), which was obtained using a maceration extraction method with 70% ethanol as the solvent. The maceration method was selected due to its ability to extract a broad range of secondary metabolites, including those that may not be heat-stable, making it an ideal approach for this study. The maceration process was performed three times to ensure maximum extraction efficiency. To prevent photochemical reactions and color changes in the extract, the samples were kept in a closed container and stored in a dark environment during extraction. Ethanol was chosen as the solvent due to its polarity, which facilitates the dissolution of both polar molecules and ions through its hydroxyl group (OH), while the alkyl group (CH₃CH₂-) can also dissolve non-polar substances, thus improving extraction efficiency (Syarif et al., 2020).

In the α-glucosidase inhibition test, several solutions were tested, including the ethanol extract of soursop leaves, control solutions of the extract, blank solution, and acarbose as a standard inhibitor. The concentrations of the ethanol extract ranged from 1000 ppm to 15.625 ppm, while the acarbose concentration series ranged from 100 ppm to 6.25 ppm. These concentration series were used to

construct a regression equation for determining the IC₅₀ value, which represents the concentration that inhibits 50% of α -glucosidase activity.

Table 1. Absorbance Measurement Results of Soursop Leaf Ethanol Extract (*Annona muricata* L.) as an α -Glucosidase Inhibitor

Concentration ($\mu\text{g/mL}$)	Absorbance	% Inhibition	Average Inhibition	% Linear Regression Equation	IC ₅₀ ($\mu\text{g/mL}$)
15.625	0.651	0.500	23.20	23.09	$y = 0.0295x + 27.458,$ $R^2 = 0.9274$
	0.498	23.50			
	0.504	22.58			
31.25	0.464	28.73	28.57		
	0.460	29.34			
	0.471	27.65			
65.5	0.457	29.80	29.29		
	0.452	30.57			
	0.472	27.50			
125	0.432	33.64	32.87		
	0.439	32.57			
	0.440	32.41			
250	0.422	35.18	36.10		
	0.413	36.56			
	0.413	36.56			
500	0.352	45.93	46.44		
	0.327	49.70			

Concentration (µg/mL)	Absorbance	% Inhibition	Average Inhibition	% Linear Regression Equation	IC ₅₀ (µg/mL)
1000	0.367	43.64			
	0.296	54.53	54.38		
	0.306	53.00			
	0.289	55.61			

The phosphate buffer solution (pH 7.0) was used in the assay, as α -glucosidase is most active at this pH level. The incubation temperature was set at 37°C, which is the optimum temperature for α -glucosidase activity. After incubation, the reaction was terminated by adding sodium carbonate (Na₂CO₃) at 0.2 M, which is commonly used to stop enzyme activity in these assays (Syarif et al., 2020). The absorbance was measured at 400 nm using a microplate reader.

The results from the ethanol extract of soursop leaves showed that the IC₅₀ value was 764.14 µg/mL. This indicates that the ethanol extract has moderate activity in inhibiting α -glucosidase, as the IC₅₀ value is relatively high. Generally, a lower IC₅₀ value indicates stronger inhibitory activity. Therefore, the soursop leaf extract does not appear to be a potent α -glucosidase inhibitor compared to other compounds, including the standard acarbose.

Table 2. Absorbance Measurement Results of Acarbose Solution as an α -Glucosidase Inhibitor

Concentration (µg/mL)	Absorbance	% Inhibition	Average Inhibition	% Linear Regression Equation	IC ₅₀ (µg/mL)
6.25	0.905	0.534	40.99	44.35	$y = 0.2422x + 41.935, R^2 = 0.983$
		0.481	46.85		
		0.496	45.19		
12.5	0.545	0.539	39.78	43.54	
		0.494	45.41		
		0.494	45.41		
25	0.490	0.430	45.86	49.28	
		0.457	49.50		
50	0.478	0.384	47.18	52.93	
		0.416	54.03		

Concentration ($\mu\text{g/mL}$)	Absorbance	% Inhibition	Average Inhibition	% Linear Regression Equation	IC ₅₀ ($\mu\text{g/mL}$)
100	0.398	0.380	56.02	66.52	
		0.131	85.52		

In comparison, the acarbose control group, which is a known α -glucosidase inhibitor, displayed a much lower IC₅₀ value of 33.30 $\mu\text{g/mL}$, demonstrating strong inhibitory activity. The inhibition percentages of acarbose increased progressively with concentration, with an average inhibition of 85.52% at the highest concentration (100 $\mu\text{g/mL}$), highlighting its potency as an inhibitor of α -glucosidase.

The relatively higher IC₅₀ value for soursop leaf extract indicates that although the extract has some inhibitory activity, it is less effective than acarbose at inhibiting α -glucosidase. This result suggests that further investigation into the active compounds in the soursop leaf extract could be valuable. Future studies could focus on isolating and identifying specific compounds responsible for the observed inhibition and evaluating their potential for development as therapeutic agents for conditions like diabetes.

In conclusion, while the ethanol extract of soursop leaves (*Annona muricata* L.) demonstrates some degree of α -glucosidase inhibitory activity, its potency is lower than that of the standard inhibitor acarbose. The study provides a basis for further research on the potential of soursop leaves in managing blood glucose levels, although its current inhibitory potential is not as high as that of established α -glucosidase inhibitors.

4. Conclusion

Based on the results of this study, it can be concluded that the ethanol extract of soursop leaves (*Annona muricata* L.) exhibits a potential, but relatively weak, inhibitory activity against the α -glucosidase enzyme. The calculated IC₅₀ value of 764.1356 $\mu\text{g/mL}$ indicates that the extract does not show a strong inhibition, especially when compared to the standard α -glucosidase inhibitor, acarbose, which had an IC₅₀ of 33.30 $\mu\text{g/mL}$. Although the ethanol extract of soursop leaves demonstrates some level of inhibitory effect, its potency is limited. This suggests that further research, possibly with different extraction methods, higher concentrations, or a combination of active compounds, may be necessary to enhance its inhibitory activity and fully explore its potential as a therapeutic agent in managing conditions such as diabetes.

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