Antidiabetic Potential of *Nypa fruticans* Fronds: Inhibition of α-Amylase, α-Glucosidase, and Glucose Absorption *In Vivo*

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Abstract

Background: Diabetes mellitus (DM) results from insulin resistance or impaired insulin secretion, leading to hyperglycemia, which, in advanced stages, causes macrovascular and microvascular complications. Effective management of postprandial hyperglycemia is critical to reducing these complications. Inhibition of carbohydrate hydrolyzing enzymes such as α-amylase and α-glucosidase offers a therapeutic approach for controlling postprandial glucose levels. While acarbose is a known inhibitor of these enzymes, its long-term use can cause gastrointestinal side effects. Therefore, alternative treatments, such as medicinal plants, are being explored. This study focuses on the antidiabetic potential of the Nipah frond (*Nypa fruticans* Wurmb), a traditional remedy, which is believed to inhibit glucose absorption and carbohydrate degradation. Methods; Nipah frond powder was extracted using ethanol through maceration. Phytochemical screening was conducted to detect bioactive compounds, and in vivo and in vitro assays were performed to evaluate antidiabetic properties. The inverted intestinal pouch technique assessed glucose absorption, while the Oral Glucose Tolerance Test (OGTT) was conducted on male Wistar rats. Enzyme inhibition assays for α-amylase and α-glucosidase were carried out to measure the extract's effectiveness in inhibiting carbohydrate degradation. Results: In vitro tests demonstrated that the ethanol extract exhibited inhibitory activity against both α-amylase and α-glucosidase enzymes, with IC50 values of 38.493 ± 0.900 ppm and 40.401 ± 0.558 ppm, respectively. In vivo studies using the everted sac technique showed that the extract reduced glucose absorption by 74.10% compared to the control group, similar to acarbose. In the OGTT, administration of 1000 mg/kg BW of Nipah frond extract significantly reduced postprandial glucose levels, with an area under the curve (AUC) comparable to glibenclamide. Conclusion: The ethanol extract of *Nypa fruticans* fronds demonstrated significant antidiabetic activity by inhibiting α-amylase and α-glucosidase, reducing glucose absorption, and suppressing postprandial hyperglycemia in vivo.

Keywords: *Nypa fruticans*, α-amylase inhibition, α-glucosidase inhibition, Postprandial Hyperglycemia, Antidiabetic Plants.

Significance | This study determined the antidiabetic potential of Nypa fruticans fronds by inhibiting carbohydrate-degrading enzymes and reducing glucose absorption.

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Introduction

Diabetes mellitus is one of the diseases that is a challenge in public health issues worldwide. The prevalence of diabetes is increasing exponentially. The World Health Organization (WHO) estimates that by 2030, diabetes will be the seventh leading cause of death worldwide. (Al-Ishaq et al., 2019). In 2019, according to the

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International Diabetes Federation (IDF), there were 463 million people (9.3%) with DM and this number will increase to 578 million (10.2%) in 2030 (Aslam *et al*., 2022). Diabetes mellitus is a metabolic syndrome caused by insulin resistance or reduced insulin secretion, which results in hyperglycemia. Hyperglycemia in advanced stages causes blood vessel damage and macrovascular disorders (atherosclerosis), microvascular (retinopathy and nephropathy), and decreased serum HDL/LDL ratio (Klisic *et al.*, 2020; Shanak *et al.*, 2019). Therefore, to prevent or slow down the manifestation of diabetes-related complications, good management of postprandial hyperglycemia is crucial to be carried out early in the management of diabetes mellitus. One way to achieve controlled postprandial blood glucose levels is to slow down glucose absorption in the intestine by inhibiting the action of specific carbohydrate hydrolyzing enzymes, namely pancreatic α-amylase and intestinal α-glucosidase and glucose transporters such as SGLT2 and GLUT2. Inhibition of the enzymes α-amylase and α-glucosidase can prevent the breakdown of carbohydrates into glucose, thereby indirectly inhibiting glucose absorption into the bloodstream. (David-Silva *et al*., 2020).

Acarbose is an α-amylase and α-glucosidase inhibitor that has been widely used, either alone or in combination with diet and insulin therapy, in diabetic patients (Bhatnagar & Mishra, 2022). Furthermore, long-term use of acarbose causes gastrointestinal disorders, such as malabsorption, flatus, diarrhea, and flatulence. These side effects occur due to increased fermentation of carbohydrates not absorbed by bacteria. Other effects include increased transaminase enzymes, hypersensitivity reactions, thrombocytopenia, edema pneumatosis cystoides intestinalis, and intestinal perforation (Fisher, 2022). Based on the previous explanation, an alternative treatment is needed with low side effects, is safe, and can be used long-term, one of which is using medicinal plants.

One of the plants that can be used as an antidiabetic is the Nipah species. The nipah plant (*Nypa fruticans*. Wurmb) is one of the plants used as a traditional antidiabetic drug (Aulia et al., 2021). The antidiabetic activity of the Nipah plant through two mechanisms, namely by delaying glucose absorption in the small intestine and inhibiting the activity of the α-glucosidase and α-amylase enzymes which play an important role in carbohydrate degradation (Nor *et al*., 2015; Yusoff *et al*., 2017). Furthermore, research by Reza *et al*. (2011) concluded that methanol extract from Nipah leaves and twigs can stimulate the residual function of pancreatic cells and increase peripheral glucose utilisation. Hence, this study aimed to examine the potential antidiabetic activity of ethanol extract of nipah fronds (*Nypa fruticans*. Wurmb) inhibiting glucose absorption through determining *in vitro* α-amylase and glycosidase inhibitory effects in the small intestine.

2. Materials and Methods

Nipah frond powder (*Nypa fruticans*. Wurmb), phosphate buffer pH 6.8, α-glucosidase enzyme (Sigma Aldrich), p-nitrophenyl-D-glucopyranoxidase (pNPG) (Sigma Aldrich), acarbose (glucobay), porcine α-amylase (Sigma Aldrich), starch (Sigma Aldrich), iodine (Sigma Aldrich), phloridzin (Sigma Aldrich).

2.1 Extraction

A total of 500 grams of nipah leaf frond powder was taken from Bugel Beach, Panjatan District, Kulon Progo Regency, Special Region of Yogyakarta (-7.5833032, 110.8173056) and determined at the Biology Laboratory of Ahmad Dahlan University with the number 190 / Lab. Bio / B / III / 2023 was extracted using 96% ethanol in a maceration vessel. The solvent was replaced every 24 hours, while the extraction process was repeated thrice. The maceration filtrate was concentrated using a rotary evaporator and then thickened using a water bath at a temperature of 60°C (Haryoto *et al*., 2023).

2.2 Rats

The rats used were healthy male Wistar strains weighing 180-250 g and aged 3-4 months obtained from the Pharmacology Laboratory of Gadjah Mada University. Before the experiment, the rats were first acclimatised for three days. The animals were placed in a well-ventilated animal transit room and given standard food and water ad libitum. The Animal Ethics Committee has approved the experimental procedure, Universitas Muhammadiyah Surakarta number: 4437/ A.1/KEPK-FKUMS/VIII/2023.

2.3 Phytochemical Screening

2.3.1 Saponin Test: 1 mL of extract solution was added to 5 mL of distilled water. The mixture was then shaken vigorously for 30 seconds, and the foam formed was observed. Confirmation of saponin compounds if foam with a 1-10 cm height is formed, which is stable for 10 minutes.

2.3.2 Tannin Test: 1.0 g of extract was dissolved in 10 mL of hot distilled water and filtered using filter paper. Five mL of the filtered results were added with five drops of 1% $FeCl_3$ solution, and colour changes were observed. The formation of a green-blue (green-black) colour indicates the presence of catechol tannins. In contrast, the formation of a blue-black colour indicates the presence of pyrogallol and triterpenoid tannins.

2.3.3 Phenolic Test: 0.5 g of the extract was dissolved in 5 mL of distilled water, then five drops of 1% FeCl3 solution were added, and the occurring colour changes were observed. Dark green or black indicates the presence of phenolic compounds

2.3.4 Flavonoid Test: 0.5 g of the extract was dissolved in 5 mL of ethanol, then a little magnesium powder and 1 mL of concentrated H2SO4 solution were added; observe the colour changes. If a formation or change in red, yellow, or orange colour occurs, it indicates a positive reaction to flavonoids.

2.3.5 Steroid/Terpenoid Test: 0.5 g of the extract was dissolved in 0.5 mL of chloroform, 0.5 mL of acetic anhydride was added, and 2 mL of concentrated H_2SO_4 was dripped carefully through the walls of the test tube. A positive triterpenoid result is formed when a brownish or violet ring is formed. A positive steroid result is formed when a bluish-green colour is formed.

2.3.6 Alkaloid Test: 1 g of extract is dissolved in 10 mL of chloroform and filtered. The filtered results are then divided into 3 test tubes, and each tube is added with five drops of Dragendorff, Wagner, and Mayer reagents. Positive results are obtained if a red precipitate is formed with the Dragendorff reagent, a white precipitate with the Mayer reagent, and a yellow precipitate with the Wagner reagent.

2.4 *Intestinal Glucose Absorption by Everted Sac Technique*

A glucose absorption test through an isolated rat jejunum pouch was studied using the method of Jadhav & Puchchakayala. After fasting overnight, a male Wistar rat (180-250 g) was sacrificed, and its abdominal wall was dissected. The jejunum was isolated from the small intestine (20 to 50 cm from the pylorus), and then the intestine was taken and inverted. The glucose absorption test was carried out using an extract solution and fractions at a dose of 1 mg/mL and 20% glucose. The inverted intestine was inserted into a tube containing a mixture of sample and glucose solutions. 0.9% physiological saline solution was added to the inside of the intestine and Then put into a water bath shaker at a temperature of 36° The solution from the inside of the intestine was taken every 10 minutes starting from the 10th, 20th, 30th, 40th, and 50th minutes then the glucose levels were analysed using the Nelson-Somogyi method. Acarbose and phloridzin (0.001 M) were used as controls.

2.5 Oral Glucose Tolerance Test (OGTT)

Oral glucose tolerance test (OGTT) of Nipah frond extract (*Nypa fruticans* Wurmb) based on the procedure carried out by Saha & Paul, (2019) which was modified. Non-diabetic rats fasted for ± 15 hours, were divided into five groups with five replications and received oral treatment. Group 1 was given CMC Na 0.5% (10 mL/kg). Group 2 was given glibenclamide 0.45 mg/kg bw (10 mg/kg) as a positive control. Groups 3, 4, and 5 were given ethanol extract of nipah fronds at doses of 250 mg/kgBW, 500 mg/kgBW, and 250 mg/kgBW, respectively. Ten minutes after single oral administration, rats were given glucose orally at a dose of 1.35 g/kgBW. Blood samples were taken from the tail tip at 0 minutes (before treatment), 30 minutes, 60 minutes, 120 minutes, and 180 minutes after glucose administration. Blood glucose levels were determined using a glucometer (Accu-Check, Roche, Mexico City, Mexico). The area under the curve (AUC) was calculated using the trapezoidal method.

2.6 In-vitro α-Amylase Enzyme Inhibition Assay

The α-amylase inhibitory activity of the samples was studied according to the method described by Magaña-Barajas et al., (2021). A total of 500 µL of extract samples with concentrations of 12.5, 25, 50, 100, and 200 ppm and acarbose with concentrations of 2, 4, 6, 8, and 10 ppm were each mixed with 500 µL of α-amylase enzyme solution and incubated at 37°C for 5 minutes. Then add 500 µL of 1% starch solution and incubate at 37°C for 10 minutes. After incubation, 100 µL of 1% iodine solution and 20 µL of 1 N HCl were added to stop the enzymatic reaction. Absorbance was measured using a UV/Vis spectrophotometer at a wavelength of 540 nm. The control solution contained the same volume of pH 6.8 phosphate buffer as a substitute for the sample. The α-amylase enzyme inhibition experiment was carried out according to the treatment in Table 1.

2.7 In-vitro α-Glucosidase Inhibition Assay

The α-amylase inhibitory activity of the samples was studied according to the method described by Magaña-Barajas et al. (2021). A total of 50 µL of extract samples with concentrations of 12.5, 25, 50, 100, and 200 µg/mL and acarbose with concentrations of 2, 4, 6, 8, and 10 µg/mL were added with 250 µL of phosphate buffer pH 6.9 and 125 µL of 5 mM PNPG, incubated at 37^{O}C for 5 minutes. After incubation, 125 µL of 0.25 U/mL enzyme solution was added and incubated for 15 minutes at 37oC. Then, 1000 µL of 200 mM Na_2CO_3 was added to stop the reaction. The absorbance of the samples was measured using an Elisa reader at a wavelength of 490 nm. The control solution contained an equal pH 6.9 phosphate buffer volume instead of the sample. The α-glucosidase enzyme inhibition experiment was carried out according to the treatment in Table 2.

2.7 Statistical Analysis

The glucose levels were determined using a linear regression equation based on the glucose standard curve, where glucose concentration is the x-axis and absorbance is the y-axis. The data of the sample absorbance measurements were entered as y in the linear regression equation to obtain the glucose levels of the samples.

Data analysis was carried out by determining the percentage of inhibition of α-amylase and α-glucosidase based on absorbance measurements. Inhibitory activity was calculated using the following equation:

$$\%\ Inhibition = \frac{(Abs\ Control - Abs\ Sample)}{Abs\ Control}\ x\ 100\%$$

The IC_{50} value was determined using a linear regression equation. This equation involves plotting the sample concentration on the x-axis and the corresponding percentage inhibition on the y-axis. The IC_{50} value was calculated using the formula IC_{50} = (50 - a)/b.

3. Results and Discussion

Table 1. α-Amylase Inhibitor Activity Test

	Volume (µL)			
	Blank	Control Blank	Sample	Control Sample
Extract	-	-	500	500
Acarbose				
α-amylase 2 U/mL	-	-	500	-
Phosphate buffer pH 6.8	1000	1000	-	-
Incubated in 37°C incubator, 10 minutes				
Starch 1%	500	500	500	500
Incubated in a 37°C incubator for 20 minutes				
Iodium 1%	100	-	100	-
Phosphate buffer pH 6.8	-	100	-	100
HCl 1 N	-	-	20	20
Measure Absorbance with UV/Vis Spectrophotometer at λ 540 nm				

Table 2. α-Glucosidase Inhibitor Activity Test Procedure

	Volume (µL)			
	Blank	Control Blank	Sample	Control Sample
Extract	-	-	50	50
Acarbose	-	-	50	50
Phosphate buffer pH 6.8	300	300	250	250
pNPG 5 mM	125	125	125	125
Incubated in a 37°C incubator for 5 minutes				
α-glukosidase enzyme 0,25 U/mL	125	-	125	-
Phosphate buffer pH 6.8	-	125	-	125
Incubated in a 37°C incubator for 15 minutes				
Na_2CO_3	1000	1000	1000	1000
Measure Absorbance with Elisa Reader At λ 490 nm				

Table 3. Phytochemical Screening of Nipah Leaf Ethanol Extract (*Nypa fruticans*. Wurmb)

Compound	**Reagent**	**Result**	**Interpretation**
Alkaloid	Mayer Dragendorf Wagner	White Brick red Yellow	+ + +
Steroid	Acetic acid anhydride + Concentrated sulfuric acid	Dark green	+
Terpenoid	Acetic acid anhydride + Concentrated sulfuric acid	Brown	+
Flavonoid	Mg powder + Concentrated hydrochloric acid	Orange	++
Fenolik	1% $FeCl_3$	Dark green	++
Saponin	Aquadest	Foam	+
Tanin	1% gelatin solution	Black Green	+

Table 5. Area under the curve (AUC) of postprandial glucose responses of normoglycemic rats in oral glucose tolerance test (OGTT).

Group	AUC
Negative Control	20793 ± 994.483^{c}
Glibenclamide	17514 ± 242.638^{a}
Extract 250 mg/kgbb	19872 ± 725.392^{ab}
Extract 500 mg/kgbb	19866 ± 1007.444^{bc}
Extract 1000 mg/kgbb	18276 ± 294.038^{a}

Table 4. Glucose levels of intestinal glucose absorption testing

Time (minute)	Glucose Level (ppm)			
	Extract	Acarbose	Phloridzin	Control
10	11.280 ± 0.581	9.547 ± 0.541	3.813 ± 0.304	51.813 ± 0.446
20	14.213 ± 1.201	12.613 ± 0.402	5.413 ± 0.326	57.013 ± 0.349
30	15.547 ± 0.906	15.147 ± 0.942	5.813 ± 0.369	59.547 ± 0.890
40	17.547 ± 0.809	19.813 ± 0.621	6.613 ± 0.335	65.013 ± 0.448
50	19.947 ± 0.706	23.547 ± 0.412	8.880 ± 0.381	77.013 ± 0.649

Table 6. The results of α-amylase and α-glucosidase enzyme inhibition assay

Group	Concentration (ppm)	α-amylase			α-glucosidase		
		% Inhibition	IC_{50}	SD	% Inhibition	IC_{50}	SD
Acarbose	0.625	20.029	4.965	0.047	0,625	4.812	0.149
	1.25	29.900			1.250		
	2.50	40.057			2.500		
	5.00	55.651			5.000		
	10.0	74.392			10.000		
Ethanol Extract Nipah Leaf Stem	12.5	35.050	38.493	0.900	43.356	40.401	0.558
	25.0	48.069			48.254		
	50.0	57.797			52.698		
	100.0	71.388			60.816		
	200.0	88.412			67.664		

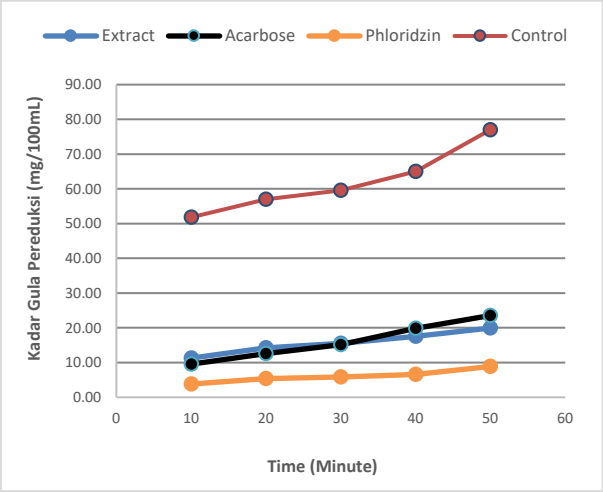

Figure 1. Effects of control, acarbose, phloridzin, and ethanol extract on intestinal glucose absorption by the everted sac technique.

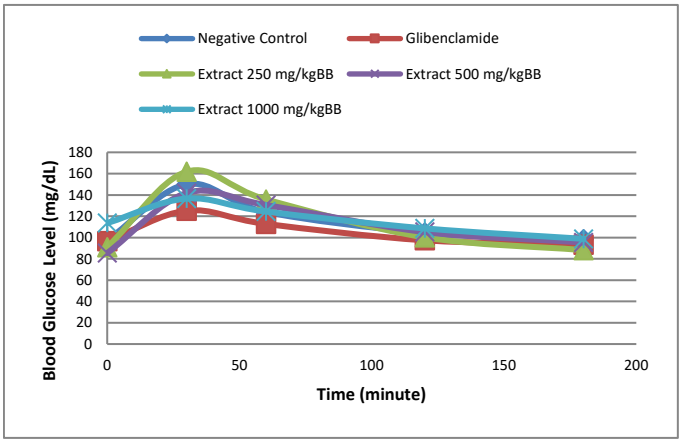

Figure 2. Increase in blood glucose levels in glucose tolerance testing.

The *Nypa fructicans* frond samples used in the study were obtained from Bugel Beach, Kulon Progo, Special Region of Yogyakarta (-7.5833032, 110.8173056). They were then determined at the Biology Learning Laboratory of Ahmad Dahlan University with the number 190/Lab.Bio/B/III/2023. The sample was extracted using the maceration method with 96% ethanol as a solvent. The maceration method was chosen to avoid heat, which is feared to damage the active compounds in the sample. About 9.8 % of Nipah frond extract was yielded.

Phytochemical screening was first carried out to identify chemical compound groups present in plant samples (Rubianti et al., 2022). Table 3 shows the results of phytochemical screening of ethanol extract of Nipah fronds.

The ethanol extract of nipah fronds was screened for various phytochemicals using specific reagents in a test tube. The test results in Table 3 showed that the extract contained alkaloids, steroids, terpenoids, flavonoids, phenolics, saponins, and tannins. Among these, flavonoids exhibited the most striking colour changes, indicating a significant presence of substantial concentrations of flavonoid compounds. Pharmacological activities associated with the flavonoid compound group include a variety of effects, especially antioxidant, anti-inflammatory, and antidiabetic properties (Maryam *et al*., 2020).

Prospandial antidiabetic testing of an ethanol extract of nipah fronds was conducted to evaluate its antihyperglycemic effects both *in vivo* and *in vitro*. *In-vivo* testing involved inhibiting intestinal glucose absorption and conducting the Oral Glucose Tolerance Test (OGTT). Meanwhile, *in-vitro* antidiabetic testing was performed enzymatically by inhibiting the enzymes α-amylase and α-glucosidase, hydrolysing carbohydrates into glucose.

Intestinal glucose absorption inhibition testing was performed using the inverted intestinal pouch technique. Two comparators were used, glibenclamide as an α-glucosidase inhibitor and phloridzin as an SGLT inhibitor. The intestinal glucose absorption test results can be seen in Figure 1 and Table 4.

Figure 1 shows the control group without adding test substances; glucose absorbed was 77.013 ppm at minute 50. However, with the presence of phloridzin, the amount of glucose absorbed decreased significantly ($p < 0.05$) by 8.880 ppm (reduced by 88.47%) compared to the control. Ethanol extract of nipah fronds showed positive results because the amount of glucose absorbed decreased significantly to 19.947 ppm ($p < 0.05$) when compared to the control (absorption decreased by 74.10%). On the other hand, acarbose showed a decrease in glucose absorption by 69.43%, with the amount of glucose absorbed at 23.547 ppm.

To confirm the activity of inhibiting intestinal glucose absorption of nipah leaf stem ethanol extract, an oral glucose tolerance test (OGTT) was conducted. The oral glucose tolerance test (OGTT) results are summarised in Table 5 and presented in Figure 2.

Figure 2 shows that in the normal control group, postprandial glucose levels caused by 1.35 g/kg glucose loading reached 150 mg/dL after 30 minutes of glucose administration. After 60 minutes, blood glucose levels dropped to 124 mg/dL and continued to fall to 98.4 mg/dL at 180 minutes after glucose loading. Administration of the extract at a dose of 1000 mg/kgBW was proven effective in suppressing postprandial hyperglycemia levels significantly ($P<0.05$) compared to the normal control group with glucose levels of 136.6 mg/dL. Glibenclamide as a comparator significantly ($p<0.05$) reduced blood glucose levels at 30 minutes with levels of 125.2 mg/dL after glucose loading. Measurement results (Table 4) Area Under Curve (AUC) of oral glucose tolerance, ethanol extract of nipah fronds at a dose of 1000 mg/kg BW (18276 ± 294.038 mg/dL) was significantly lower than normal controls (20793±994.483 mg/dL) ($p<0.05$) and comparable to glibenclamide as a comparator (17514±242.639 mg/dL). Therefore, it can be concluded that the administration of Nipah frond extract at a dose of 1000 mg/kgBW showed a comparable effect to glibenclamide at a dose of 0.45 mg/kgBW in suppressing postprandial hyperglycemia. The similarity of nipah leaf stem extract with phloridzin and acarbose makes it possible that Nipah leaf stem extract has an inhibitory effect on intestinal glucose transporters due to the pharmacological activity of phloridzin as a blocker of the rate of intestinal glucose absorption through inhibition of sodium/glucose-related transporters (SGLT) located in the small intestine mucosa (Ouassou et al., 2018).

The inhibitory activity of ethanol extract of nipah fronds against α-amylase and α-glucosidase enzymes was measured using starch and p-nitrophenyl-α-d-glucopyranoside (pNPG) as a substrate, respectively. The inhibitory activity of both enzymes was then compared with acarbose as a comparator. Acarbose, as an inhibitor of α-glucosidase and α-amylase enzymes, can delay the carbohydrate digestion process by inhibiting the breakdown of carbohydrates into glucose so that it can slow down the absorption of glucose in the small intestine and reduce blood glucose concentrations after meals (Gong et al., 2020). The test results showed that Nipah leaf stem extract can inhibit the activity of α-amylase and α-glucosidase enzymes, as seen in Table 6 and Table 7. Nipah leaf stem extract provides the inhibitory activity of α-amylase enzyme with an IC_{50} value of 38.493 ± 0.900 ppm, while acarbose provides an IC_{50} value of 4.965 ± 0.047 ppm. In the inhibition test of α-glucosidase enzyme activity, Nipah leaf stem extract provides inhibitory activity with an IC_{50} value of 40.401 ± 0.558 ppm, while acarbose provides an IC_{50} value of 4.812 ± 0.149 ppm. This proves that ethanol extract can significantly inhibit the activity of α-amylase and α-glucosidase enzymes. Inhibition of the enzymes α-amylase and α-glucosidase can delay the degradation of complex sugars into glucose, which helps delay glucose absorption in the

small intestine, ultimately lowering postprandial blood sugar levels (Yen *et al*., 2021).

The postprandial antihyperglycemic activity of nipah leaf ethanol extract is related to the content of compounds such as saponins and flavonoids. Saponin compounds can suppress the transfer of glucose from the stomach to the small intestine and inhibit the transport of glucose and fluid in the boundary membrane (Mahomoodally *et al*., 2012). Flavonoid compounds act as competitive inhibitors by binding to the enzyme's active site so that the enzyme cannot convert the substrate into glucose. In addition, flavonoids can inhibit glucose absorption by inhibiting glucose transporters such as SGLT2 and GLUT2, so that they can reduce glucose reabsorption by increasing glucose excretion in the urine and glucose absorption in the small intestine (Niisato & Marunaka, 2023).

4. Conclusion

The results showed that ethanol extract of nipah fronds reduces postprandial hyperglycemia and delays the absorption of food glucose in the intestine through inhibition of intestinal glucose transporters located on the apical side of small intestinal enterocytes. In addition, ethanol extract of nipah fronds inhibits the enzymes α-amylase and α-glucosidase with IC_{50} values of 38.493±0.900 ppm and 40.401±0.558 ppm, respectively. These findings are potentially crucial for further research on identifying active compounds responsible for.

Author contributions

All authors made equal contributions to the study design, statistical analysis, and drafting of the manuscript. The corresponding author, along with the co-authors, reviewed and approved the final version of the article prior to submission to this journal.

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Competing financial interests

The authors have no conflict of interest.

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