Phytochemistry and Antidiabetic Activity from the Leaf Midrib of *Nypa fruticans* Wurm.

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Abstract: *This study aims to determine the antidiabetic activity of* n-*hexane and ethanol extracts of* Nypa fruticans *leaf sheaths. The ethanol extract, with the most active antidiabetic properties from the* n-*hexane extract, was characterized using GC-MS. The total phenol, total flavonoid, and antioxidant properties were determined, and then the ethanol extract was fractionated. An antidiabetic activity test was carried out using the glucose tolerance method in male Swiss Webster mice. The results showed that subfraction A (the effect of fractionation of ethanol extract) was the most capable of reducing blood sugar levels, namely 247.67 mg/dL in 30 min (the activity is 98.67% compared to glibenclamide). The ethanol extract can reduce blood glucose levels by 203.34 mg/dL at 30 min (81.01% compared to glibenclamide). The results of total phenolic and total flavonoid content tests for the ethanol extract were obtained at 18.0349 mg gallic acid equivalents/g and 2.8309 mg quercetin equivalents/g, respectively. On the other hand, the* IC_{50} *antioxidant activity of the extract is 417.4399 ppm. Characterization using GC-MS on the extract showed that the dominant phytoconstituent in the extract is 4-methoxy-6-(2-propenyl)-1,3-benzodioxole, with a composition of 63.45%.*

Keywords: *antidiabetic;* Nypa fruticans*; glucose tolerance*

■ INTRODUCTION

Diabetes mellitus remains a significant global health challenge. It is estimated that 117 individuals globally suffer from diabetes, which is a condition characterized by persistent metabolic disorders and elevated levels of blood sugar [1]. Diabetes mellitus can cause various complications, both macrovascular and microvascular, such as cardiovascular disorders and hypertension [2], insulin resistance, obesity, and dyslipidemia [3]. Until now, the need for drugs is substantial, and sometimes drugs are individualistic, so the search for drugs will continue.

Plants are a source of natural medicine, and one of the plants is *Nypa fruticans* Wurmb. *N. fruticans* or Nipa plant is a type of palm that grows in mangrove forest environments or tidal areas near the edge of the sea. Nipa palms have stems submerged under a layer of mud that spreads underground with a stem thickness of approximately 60 cm [4]. It was reported that vinegar derived from the plant can reduce postprandial hyperglycemia in type 2 diabetes patients with a moderate glycemic index [5]. In a previous study, giving an aqueous vinegar extract at a dose of 1,000 mg/kg to adult male Sprague-Dawley rats significantly reduced blood glucose levels [6-7]. The crude extract of fruit skin, fruit flesh, seed coat, and whole ripe fruit of the plant extracted with water and ethanol can inhibit the xanthine oxidase that causes gout. The crude extract contains phenolics, flavonoids, terpenoids, and steroids. The ethanol extract of the seed coat contains high phenolics and flavonoids, which have potent antioxidant activity [8]. Besides that, Indonesia has nipa palm forests, including vast mangrove forests reaching 3.2 million hectares, which can later be used as a source of medicines, especially for diabetes [4]. Therefore,

research on the antidiabetic activity of the leaf midrib extract of the plant that grows in Aceh will be carried out. The leaf midribs were tested on Swiss Webster male mice using the glucose tolerance method.

■ EXPERIMENTAL SECTION

Materials

The sample used in this research was the leaf midribs of the plant taken in West Aceh, Aceh, Indonesia. The plant was identified at the Herbarium Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala. The results of the determination showed that the *N. fruticans* belonging to the Arecaceae belonged to the species *Nypa fruticans* Wurmb. The indicator animal used was the Swiss Webster mice purchased from the Faculty of Veterinary Medicine Laboratory, Universitas Syiah Kuala. Meanwhile, other chemicals used included glibenclamide (Indofarma), glucose, gallic acid (Sigma-Aldrich), quercetin (E-Merck), 1,1-diphenyl-2-picrylhydrazil (DPPH, Sigma-Aldrich), aluminum chloride ($AlCl_3$, E-Merck), Folin-Ciocalteu reagent (Sigma-Aldrich), and sodium carbonate (Na_2CO_3, E-Merck).

Instrumentation

The mass spectra were analyzed utilizing a Shimadzu gas chromatography-mass spectrometry (GC-MS) QP 2010 Ultra instrument. The column chromatography process was conducted using silica gel G60 (70–230 mesh Merck) (Sigma-Aldrich). Precoated silica gel G60-F254 (Sigma-Aldrich) was utilized for thin-layer chromatography (TLC) examination, and diabetes mellitus test (glucose) used Easy Touch GCU strip test type. Then, a 96-well microplate (IWAKI), flat microplates with several "wells" were used as small test tubes.

Procedure

Phytochemical screening

The procedures performed to analyze phytochemicals, specifically terpenoids, steroids, phenolics, flavonoids, alkaloids, and saponins, are detailed in Phytochemical Methods, a comprehensive manual on contemporary plant analysis methodologies. This approach enables rapid identification of secondary metabolites with high precision [9].

Preparation of extracts from leaf midrib of N. fruticans *and subfraction*

The leaf midrib of the plant, which was as much as 15 kg, was cleaned, dried, crushed, weighed, and macerated with *n*-hexane solvent for 2 × 24 h. After filtration, the obtained filtrate was evaporated with a vacuum rotary evaporator, and a concentrated *n*-hexane extract of 7.0427 g (0.36%) was obtained. Then, the residue was macerated with ethanol solvent, in the same way as with *n*-hexane solvent, to obtain a concentrated ethanol extract of 60.325 g (3.1%). Furthermore, both extracts were tested for their antidiabetic activity at 50 mg/kg BW concentrations using the glucose tolerance method, and it was reported that ethanol extract was more active than *n*-hexane extract. Then, the ethanol extract was characterized using GC-MS and fractionated by column chromatography. The subfractions resulting from separating the ethanol extract by column chromatography were 1.3067 g of subfraction A and 24.7601 g of subfraction B. Both subfractions were tested for antidiabetic activity at 50 mg/kg BW.

Fractionation of ethanol extract from the leaf midrib of the plant

As much as 30.05 g of ethanol extract of the leaf midribs of the plant was separated by gravity column chromatography (GCC), using the stationary phase was silica gel 60 (0.2–0.5 mm) and the mobile phase was a mixture of *n*-hexane and ethyl acetate, with increasing polarity successively namely *n*-hexane (100%); *n*-hexane: ethyl acetate (90:10; 80:20; 70:30; 60:40; 50:50; 40:60; 30:70; 20:80; 10:90), and ethyl acetate (100%). Eluate was collected every 100 mL, and column chromatography results obtained 146 fractions. The fractions with the same stain pattern were combined, evaporated, and two combined subfractions were obtained, namely subfraction A (1-126) and subfraction B (127-146). The profiles of the subfractions resulting can be seen in Table 1.

Table 1. Subfractions of the ethanol extract (subfractions A and B)

Subfraction	Weight (g)	Color
A (1-126)	1.3067	Orange
B (127-146)	24.7601	Chocolate

Preparation of 1% CMC-Na

As much as 1 g of sodium carboxymethylcellulose (CMC-Na) is sprinkled in 20 mL of warm distilled water for 15 min until the CMC-Na swells and is crushed until homogeneous. The homogeneous CMC-Na was put into a 100 mL volumetric flask and added with warm distilled water up to the mark.

Preparation of glibenclamide solution (positive control)

As much as 0.005 g of glibenclamide was dissolved in 50 mL of 1% CMC-Na. The dose of glibenclamide given to the mice is 0.45 mg/kg BW of mice given orally [10].

Preparation of bioassay test

The test preparation (*N. fruticans* leaf midrib extract) was suspended in a 1% w/v CMC-Na solution with a concentration of 0.5% w/v. The test preparations were prepared by weighing each test preparation as much as 0.025 g and suspended in 0.5 mL of CMC-Na. The dose given was 50 mg/kg BW of mice [11].

Preparation of glucose solution

A total of 2.5 g of glucose was dissolved in 5 mL of distilled water. The dose of glucose is as much as 3 g/kg BW in mice [12].

Antidiabetic activity testing with the glucose tolerance method

Prior to the start of the experiment, the mice had a seven-day period of acclimatization in laboratory circumstances, during which they were provided with appropriate food and water [13]. After a period of seven days, mice that were in a healthy state were chosen based on their consistent or increasing body weight and absence of any aberrant behavior. Furthermore, the mice were categorized into three groups, with each group including three mice. Mice in group I were administered CMC-Na (1%) as a negative control to evaluate the antidiabetic activity. Mice in group II were administered the conventional medication, glibenclamide, orally at a dosage of 0.45 mg/kg BW, serving as a positive control. Mice in group III-VI were administered *n*-hexane extract, ethanol extract, subfraction A, and subfraction B, respectively, at a dosage of 50 mg/kg BW (effective dose) [11].

After the mice were fasted for 20–24 h and weighed, their fasting blood glucose levels were measured. These mice were treated for negative control, positive control, and extracts/subfraction. After 30 min, all groups of mice were given glucose orally at a dose of 3 g/kg BW. Furthermore, the mice's blood glucose levels were recorded at the 30^{th}, 60^{th}, 90^{th}, and 120^{th} min after glucose loading.

Blood sugar measurement

The blood sugar measurement was performed by cleansing the tails of mice using a moist cotton swab to eliminate any dirt, followed by the application of 70% (v/v) alcohol to the tails. Blood was collected from the lateral tail vein by making an aseptic incision approximately 1–2 mm from the tail tip without the use of anesthesia. The initial blood droplet was extracted, followed by the subsequent droplet being applied to the One Touch Horizon strip.

Determination of total phenolic content

The determination of total phenolic content was carried out using the Folin-Ciocalteu method [14] with slight modifications [15]. As much as 10 mg of the extract was dissolved in 100 mL methanol (100 ppm). Then 50 µL of the solution was put into the well and successively diluted so that concentrations of 100, 80, 60, 40, 20, 10, and 1 ppm were obtained. Each solution was added with 50 µL of 10% Folin-Ciocalteu solution, then stirred, and allowed to stand for 5 min, then added 100 µL of 5% Na_2CO_3. In the same way, the sample treatment was carried out on the standard solution. Standard gallic acid with various concentrations, namely 100, 50, 25, 12.5, 6.25, 3.1, and 1.5 ppm. The mixture was incubated at 40 ° for 1 h. The total phenolic content was determined by using a microplate reader to read the absorbance at a wavelength of 765 nm. The phenol concentration in the extract was calculated using the gallic acid calibration

regression equation. Total phenolic content is expressed in gallic acid equivalent (mg GAE/g extract).

Determination of total flavonoid content

The total amount of flavonoids in the extract used the colorimetric $AlCl_3$ method. A total of 25 mg of the extract was dissolved in 50 mL of methanol (500 ppm). A total of 50 µL of the solution was put into the well with concentrations of 500, 250, 125, 50, 25, 12.5, and 5 ppm. The solution was added 0.1 µL $AlCl_3$ 10%. Then, the same thing was done for standard quercetin with various concentrations, namely 25, 12.5, 6.25, 3.1, and 1.5 ppm. The solution was incubated at room temperature for 30–60 min.

Using a microplate reader, the total flavonoid content was determined by reading the absorbance at a wavelength of 434 nm. The concentration of flavonoids in the extract was calculated using the regression equation of the quercetin calibration. Total flavonoid content is expressed by quercetin equivalent (mg QE/gr extract) [15].

Antioxidant activity

An antioxidant activity test was carried out using a microplate reader with the DPPH method at a wavelength of 516 nm. The plate consists of rows A-H totaling 12 wells. Each 10 mg sample was dissolved in 10 mL of methanol as mother liquor (1000). Dilutions were made with 1000, 800, 600, 400, 200, 100, and 10 ppm concentrations. Each row (A-H) was filled with 100 µL, and then 80 µL of DPPH was added with a concentration of 80 ppm. The mixture was left in a dark place for 30 min, and then the absorbance was measured. For the negative control, 80 µL of DPPH 80 ppm was used, while for the blank (methanol) was used. Ascorbic acid was used as a positive control with the same treatment as the sample treatment with variations in the concentration of 100 (A), 50 (B), 25 (C), 12.5 (D), 6.25 (E), and 3.125 ppm (F) as percent attenuation (% inhibition) with the Eq. (1)

$$\%\ \text{inhibition} = \frac{\text{control}_{abs} - \text{sample}_{abs}}{\text{control}_{abs}} \times 100\% \quad (1)$$

Furthermore, the calculation results are entered into the regression equation. The x-axis or abscissa is the extract concentration (ppm), and the y-axis or ordinate is the percentage of radical scavenging. Inhibition percentage data is used to find the value of inhibition concentration (IC_{50}) in ppm.

Calculations with SPSS

Statistical analysis was carried out using the Statistical Product and Service Solution (SPSS) Program to compare the optimum concentration of extracts and subfractions with glibenclamide. Analysis of variance was performed using a one-way ANOVA. Post hoc analysis procedure and significant differences ($p < 0.05$) and ($p < 0.01$) were used by Tukey [16].

■ RESULTS AND DISCUSSION

Phytochemical Test Results

The content of secondary metabolites in the hexane and ethanol extracts of the plant can be seen in Table 2. From Table 2, it was shown that the results of the phytochemical test of the *n*-hexane extract of the plant did not contain secondary metabolites or it contained a trace number of secondary metabolites, so they were not detected (negative value). Meanwhile, the ethanol extract contains flavonoids with a strong intensity with HCl and Mg reagents. The appearance of a red color in the ethanol extract when reacted with Liebermann Burchard's reagent indicated a positive presence of terpenoids and a green or blue color for steroids. The phenolic compounds contained in the ethanol extract are characterized by the appearance of a dark green color caused by the formation of a complex of Fe^{3+} ions with phenol groups. *N. fruticans* extract contains phenolic and flavonoid compounds that exhibit antidiabetic activity [6].

Characterization of the Ethanol Extract of *N. fruticans* Using GC-MS

The characterization of chemical content in the ethanol extract was conducted using GC-MS. After adjusting with Library Mass Spectrometry Data, seven compounds were obtained. However, only 2 dominant compounds have a large composition and also had a similarity of above 80% (as shown in Table 3).

In Table 3, there are two major compounds contained in the ethanol extract of the leaf midrib, namely 4-methoxy-6-(2-propenyl)-1,3-benzodioxole and

Table 2. The content of secondary metabolites of fresh leaf midrib, *n*-hexane, and ethanol extract of the plant

Secondary metabolite	Midrib (Fresh sample) *n*-hexane	Midrib (Fresh sample) ethanol	*n*-Hexane extract	Ethanol extract
Steroids				
• Liebermann-Burchard reagent	−	+	−	+
Terpenoids				
• Liebermann-Burchard reagent	−	−	−	+
Saponin				
• Shaking with water	−	+	−	+
Flavonoids				
• HCl and Mg	−	+	−	+
Phenolic				
• $FeCl_3$ reagent	−	+	−	+

Note: (+): Positive for the presence of secondary metabolites
(−): Negative for the presence of secondary metabolites

Table 3. The compounds contained in the ethanol extract of the plant using GC-MS

Similarity (%)	Retention time (min)	Name	Structure	Areas
89.5	22.601	4-Methoxy-6-(2-propenyl)-1,3-benzodioxole		63.45
82.1	31.267	Methyl hexadecanoate		26.33

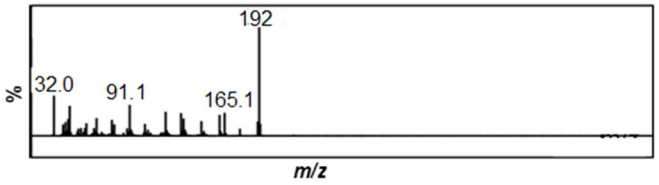

Fig 1. MS spectrum of 4-methoxy-6-(2-propenyl)-1,3-benzodioxole

methyl hexadecanoate with a similarity of 89.5 and 82.1%. The compound 4-methoxy-6-(2-propenyl)-1,3-benzodioxole, is a phenylpropanoid compound that has antidiabetic activity [17-18], conducted a study comparing three plants as antidiabetics found that plants containing 4-methoxy-6-(2-propenyl)-1,3-benzodioxole had the highest antidiabetic activity. Meanwhile, methyl hexadecanoate is a compound of the palmitic acid group. Phenylpropanoid is a secondary metabolite which is included in the phenol group. From literature searches, phenol compounds have antidiabetic activity [19].

The presence of the compound 4-methoxy-6-(2-propenyl)-1,3-benzodioxole in this ethanol extract is strengthened by the data resulting from the characterization of the leaf midrib with MS as shown in Fig. 1.

The mass spectrum of the compound has a peak at *m/z* 192. This fragmentation pattern is very identical to the mass spectrum in previous studies, which has a fragmentation pattern at *m/z*: 192, 177, 161, 147, 119, 77, 65, and 35 [19]. The fragmentation pattern is presented in Fig. 2. The fragmentation pattern from the mass spectrum of the compound 4-methoxy-6-(2-propenyl)-1,3-benzodioxole can be observed that the termination of the $-OCH_3$ at *m/z* 192 produces a compound with *m/z* 161. Furthermore, the compound at *m/z* 161 undergoes $-OCH_2$ cleavage to produce a compound at *m/z* 131. The compound at *m/z* 131 undergoes CO cleavage to produce a compound with *m/z* 103. Fragmentation of

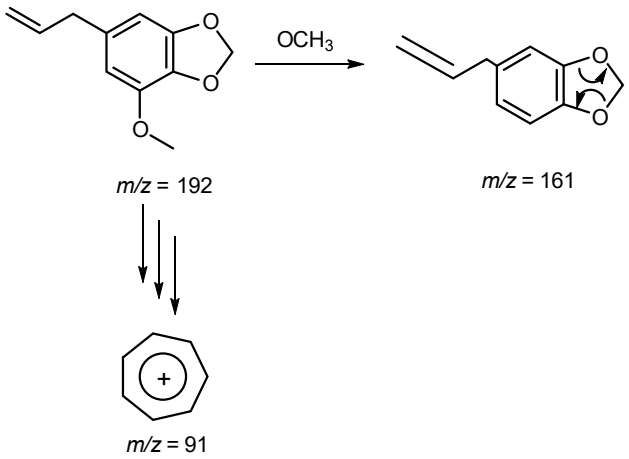

Fig 2. Fragmentation pattern of 4-methoxy-6-(2-propenyl)-1,3-benzodioxole

compounds with *m/z* 192 can also undergo CH_3 cleavage to form compounds with *m/z* 177, which then undergo OCH_2 cleavage to form compounds with *m/z* 147. Compounds with *m/z* 147 undergo CO cleavage to form compounds with *m/z* 119. This compound undergoes CO cleavage to form a compound with *m/z* 91. Furthermore, the compound with *m/z* 91 undergoes CH_2 cleavage to form a compound with *m/z* 77, and this compound with *m/z* 77 undergoes C cleavage to form a compound with *m/z* 65. Compounds with *m/z* 65 undergo CH_2 cleavage to form compounds with *m/z* 35 [19].

Antidiabetic Activities

The antidiabetic test was subjected to *n*-hexane extract, ethanol extract, subfraction A, and subfraction B of leaf midribs, negative control (CMC-Na), and positive control (glibenclamide) in mice. Blood sugar levels were measured using a glucometer in male Swiss Webster mice that had been induced diabetes. As a result, it can be seen in Fig. 3. In Fig. 3, it was shown that the group of mice that were given glibenclamide at the initial measurement of glucose levels was 100 methyl hexadecanoate mg/dL, then in the 30th min after giving glucose, blood glucose levels rose to 166.67 mg/dL. Measuring blood glucose in mice at 60, 90, and 120 min, it will be measurable at 150, 100, and 86.67 mg/dL, respectively.

In the group of mice that were given the ethanol extract, the initial blood glucose level was 108.67 mg/dL. In the 30th min after administration of the glucose solution, the blood glucose level rose to 214.33 mg/dL. In the 60th min, the blood glucose levels of the mice dropped to 185.67 mg/dL. Next, the blood glucose levels of mice fell to 141 mg/dL in the 90th min and finally to 112.67 mg/dL in the 120 min.

In the group of mice that were given subfraction A, the initial blood glucose level was 67.66 mg/dL. Then, 30 min after administration of the glucose solution, the blood glucose level rose to 170 mg/dL. However, at 60 min, the mice's blood glucose levels fell to 150.67 mg/dL. Next, the blood glucose levels of the mice fell to 146.67 mg/dL at 90 min; and finally, to 132 mg/dL at 120 min.

In the group of mice that were given subfraction B, the initial blood glucose level was 107 mg/dL. Then, 30 min after administration of the glucose solution, the blood glucose level rose to 250.33 mg/dL. In the 60th min, the blood glucose levels of the mice dropped to 273.67 mg/dL. Next, the blood glucose levels of the mice fell to 202.33 mg/dL in the 90th min; and finally, to 148 mg/dL at 120 min.

In the group of mice that were given the *n*-hexane extract, the initial blood sugar level was 104.67 mg/dL. Then, 30 min after administration of the glucose solution, the blood glucose level rose to 294 mg/dL. In the 60th min, the mice's blood glucose levels dropped to 214.67 mg/dL.

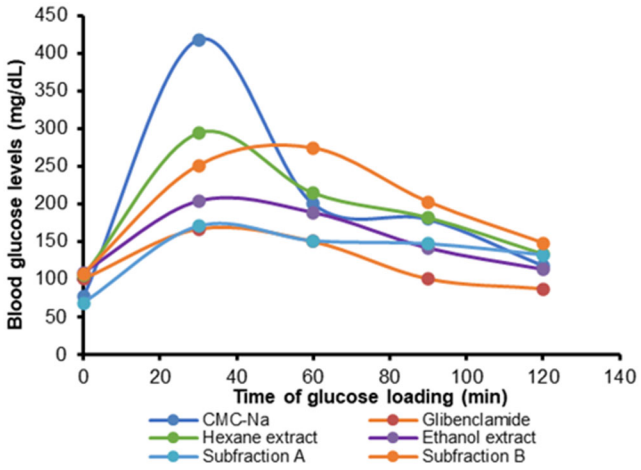

Fig 3. Decrease/increase in the average blood glucose of mice given: *n*-hexane extract, ethanol extract, subfraction A, and subfraction B of leaf midribs, negative control (CMC-Na), and positive control (glibenclamide) concerning time

In the 90th min, the mice's blood glucose levels fell to 182 mg/dL, and blood glucose levels fell to 133.67 mg/dL at 120 min.

In the group of mice that were given CMC-Na (negative control), the initial blood sugar level was 76.33 mg/dL. Then, 30 min after administration of the glucose solution, the blood glucose level rose to 417.67 mg/dL. In the 60th min, the blood glucose level of the mice dropped to 200 mg/dL. In the 90th min, the blood glucose level of the mice fell to 179.33 mg/dL, and the blood glucose level fell to 118 mg/dL in the 120 min.

The activity of lowering blood glucose levels of mice in *n*-hexane extract was not as strong as glibenclamide and relatively smaller than that of ethanol extract. This is based on the phytochemical tests that have been carried out, which show that the *n*-hexane extract does not contain secondary metabolites in sufficient amounts. The ethanol extract contains terpenoids, saponins, and flavonoids, which can reduce blood glucose in mice. Terpenoid compounds, flavonoids, and saponins have been known to have antidiabetic activity [20]. Flavonoids are compounds that have the ability to lower blood glucose levels by preventing the absorption of glucose and inhibiting the activities of α-amylase, an enzyme that breaks down disaccharides and polysaccharides into glucose in the intestine. This mechanism helps to stabilize plasma glucose levels and minimize fluctuations, making flavonoids effective antidiabetic medicines [21].

Furthermore, to find out which extract or subfraction was the most active, we reduced the antidiabetic activity of the negative control (CMC-Na) with the antidiabetic activity of *n*-hexane extract, ethanol extract, subfraction A, and subfraction B. The results of reducing blood glucose levels in negative control with blood glucose levels of mice given extracts and subfractions are shown in Fig. 4.

In Fig. 4, the curve reduction between blood glucose levels of negative control mice and blood glucose levels of mice given *n*-hexane extract, ethanol extract, subfraction A, and subfraction B showed a decrease in blood glucose levels of mice that varied. At 30 min after loading, ethanol extract can reduce the blood glucose levels of mice by 203.34 mg/dL. In the 60th min, it was as much as 14.33 mg/dL; in the 90th min, it was 38.33 mg/dL, and in the 120th min, it was 5.33 mg/dL. The *n*-hexane extract at 30 min was able to reduce mice blood glucose levels by 123.67 mg/dL, while at 60, 90, and 120 min, it worked as an antagonist, causing an increase in mice blood glucose each by 14.67, 2.67, and 15.67 mg/dL, respectively.

Subfraction A at 30 min after loading can reduce blood glucose levels of mice by 247.67 mg/dL, by 60th min by 49.33 mg/dL, by 90th min by 32.66 mg/dL, and by 120 min can increase blood glucose levels in mice by 14 mg/dL. Whereas in subfraction B at 30 min, it can reduce blood glucose levels by 167.34 mg/dL, at 60, 90, and 120 min, it can increase blood glucose levels by 73.67, 23, and 30 mg/dL, respectively. To see the differences in blood glucose lowering activity in Swiss Webster male mice between glibenclamide, hexane extract, ethanol extract, subfraction A, and subfraction B, SPSS analysis was used. The analysis used one-way ANOVA Post hoc analysis using Tukey, and the results were displayed as shown in Table 4.

Based on the results of the analysis with SPSS and Tukey's test, it can be seen that the blood sugar-lowering activity of the *n*-hexane extract was significantly different from that of glibenclamide at 30 ($p < 0.05$), 60 ($p < 0.1$), 90 ($p < 0.05$), and 120 min ($p < 0.05$). The ethanol extract was significantly different from glibenclamide at

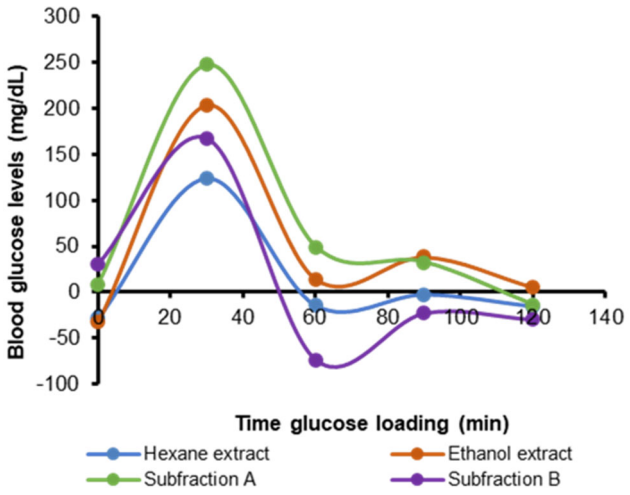

Fig 4. Reduction curve of decrease/increase in blood glucose levels of control mice (CMC-Na) with blood glucose levels of mice given n-hexane extract, ethanol extract, subfraction A, and subfraction B

Table 4. Comparison of the decrease in blood glucose levels of mice given *n*-hexane extract, ethanol extract, subfraction A, and subfraction B with glibenclamide

Group	Mice blood glucose levels (mg/dL)											
	30 min			60 min			90 min			120 min		
	Avg	SD	P	Avg	SD	P	Avg	SD	P	Avg	SD	P
n-hexane extract	294.00	48.69	0.012*	214.67	50.08	0.089^	182.00	25.94	0.009*	133.67	14.04	0.017*
Ethanol extract	214.33	21.22	0.032*	185.67	41.95	0.215	141.00	29.20	0.097^	112.67	8.38	0.061^
Subfraction A	170.00	50.74	0.918	150.67	9.50	0.911	146.67	10.40	0.011*	132.00	24.24	0.052^
Subfraction B	250.33	105.60	0.246	273.67	44.06	0.008*	202.33	11.59	0.001*	148.00	17.69	0.010*
Glibenclamide	166.67	14.43	-	150.00	2.00	-	100.00	15.00	-	86.67	15.27	-

*: Significantly different from subfraction A ($p < 0.05$)
^: Significantly different from subfraction A ($p < 0.1$)

30 ($p < 0.05$), 90 ($p < 0.1$), and 120 min ($p < 0.1$), not significantly different at 60 min. Subfraction A was significantly different from glibenclamide at 90 ($p < 0.05$) and 120 min ($p < 0.1$), not significantly different at 30 and 60 min. Subfraction B was significantly different from glibenclamide at 60 ($p < 0.05$), 90 ($p < 0.05$), and 120 min ($p < 0.05$), not significantly different at 30 min.

The strength of reducing blood sugar levels of *n*-hexane extract, ethanol extract, subfraction A, and subfraction B in mice, is a comparison of the activity of reducing blood sugar levels of each sample with the activity of reducing blood sugar levels of mice given glibenclamide multiplied by 100% [22]. The result of lowering power mice blood sugar compared to glibenclamide can be seen in Table 5.

Based on Table 5, it can be seen that subfraction A has the ability to lower blood sugar levels in mice close to glibenclamide at the 30th (98.67%) and the 60th min (98.66%). Furthermore, the ethanol extract has the power to lower blood sugar levels in mice by 81.01% (compared to glibenclamide) and subfraction B (66.66% compared to glibenclamide in 30 min). For the hexane extract, its activity was below 50% in all min of measurement, as well for ethanol extract and subfraction B, the activity was below 50% at 60 min, and for all samples, at 90 and 120 min, the activity was below 50% compared to glibenclamide.

Total Phenolic Content

The results of absorbance measurements at several concentrations of gallic acid were 50, 25, 12.5, 6.25, 3.1, and 1.5 ppm, obtaining a direct association between absorbance and a concentration of 0.9986. The calculation results indicate that the intercept value is 0.114, and the slope value is 0.048. Therefore, the standard curve equation can be expressed as y = 0.048x + 0.114. The equation presented in Fig. 5 is utilized for the quantitative assessment of the total phenolic content

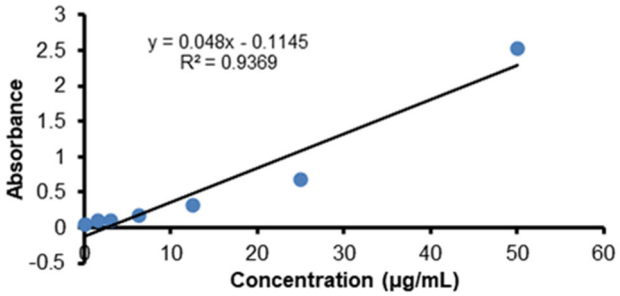

Fig 5. Gallic acid calibration curve at a wavelength of 765 nm

Table 5. Comparison of the percentage activity of each test preparation to a positive control (glibenclamide)

Test Preparation	Time for measurement of blood glucose mice (min)			
	30 (%)	60 (%)	90 (%)	120 (%)
n-hexane extract	49.27	−29.34	−3.36	−50.01
Ethanol extract	81.01	28.66	48.31	17.01
Subfraction A	98.67	98.66	41.16	−44.68
Subfraction B	66.66	−147.34	−28.99	−95.75

of the ethanol extract. This content is measured in relation to gallic acid.

The total phenolic content in the ethanol extract was obtained by inserting the absorbance value of the sample into the gallic acid standard curve. Based on this calculation, the average total phenolic content of the ethanol extract from the leaf midribs was found to be 18.0349 ± 0.1779 mg GAE/g.

Total Flavonoid Content

The results of measuring the absorbance of quercetin at several concentrations were 25, 12.5, 6.25, 3.1, and 1.5 µg/mL, obtaining a direct association between absorbance with a concentration of 0.9951. The calculation results show that the intercept value is 0.0309, and the slope value is 0.0142. Therefore, the standard curve equation is y = 0.0142x + 0.0309. This equation is used in the quantitative analysis of measuring the total flavonoid content as quercetin in the ethanol extract, shown in Fig. 6. The total flavonoid content in the ethanol extract was determined by inserting the absorbance value of the sample into the standard quercetin curve. Based on the calculation, the average total flavonoid content of the ethanol extract from the leaf midribs was found to be 2.83 ± 0.4225 mg QE/g.

Antioxidant Activity

Testing the antioxidant activity of the leaf midribs extract was made in several concentrations, namely 1000, 800, 600, 400, 200, 100, and 10 µg/mL. Next, measurements were taken on the sample, and the absorbance was obtained from each concentration in the sample. Then, the percentage inhibition was performed and calculated. Then, plot the percentage of inhibition and concentration data so that the regression equation is obtained, as presented in Fig. 7.

In this study, the regression equation obtained from the ethanol extract of the midribs of leaf y = 0.0707x + 20.487 with a correlation coefficient (R^2) of 0.919. Then, from the regression equation, it can be calculated about the IC_{50} value of the sample. The amount of antioxidant activity is expressed by the IC_{50} value, which is the concentration of the sample solution that can reduce DPPH by 50%. In general, the lower the IC_{50} value obtained, the higher the antioxidant activity [23].

From the calculation results, the IC_{50} value obtained from the ethanol extract of the leaf's midrib was 417.4399 µg/mL, and ascorbic acid as a positive control was 4.81 µg/mL. Based on these results, it can be seen that the ethanol extract has weak antioxidant activity. However, research done by Putri [24] shows that the antioxidant activity of the methanol extract of *N. fruticans* leaves has an IC_{50} value of 17.72 µg/mL.

Next, the groups of compounds that provide antioxidant activity are phenolic and flavonoid compounds [25]. The chemical structure, number, and position of the hydroxyl groups determine the difference in the antioxidant activity of compounds [26]. Phenolic and flavonoid compounds act as antioxidants because they have a hydroxyl group attached to a carbon with a conjugated double bond, so the hydroxyl group can easily donate hydrogen atoms to free radicals. Thus, the more the content of phenolic and flavonoid compounds in a plant, the greater the antioxidant activity [27].

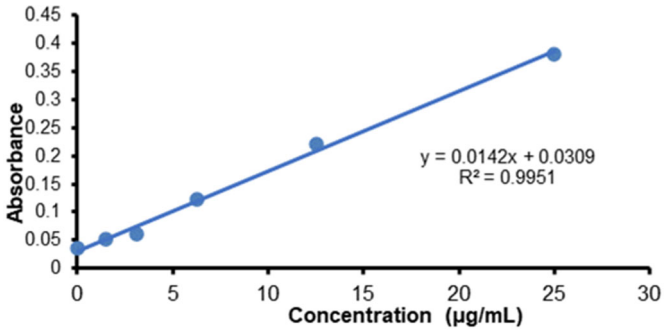

Fig 6. Quercetin calibration curve at a wavelength of 434 nm

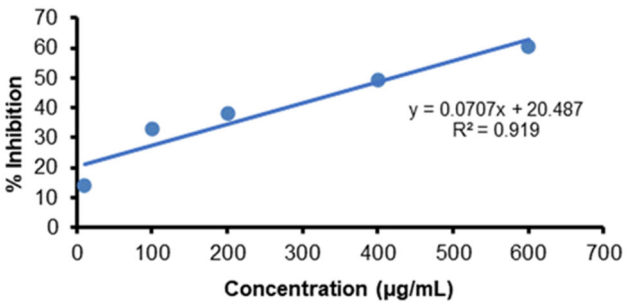

Fig 7. The regression equation for ethanol extract of *N. fruticans* leaf sheaths

■ CONCLUSION

The results of the phytochemical screening showed that the ethanol extract of the leaf midrib of *N. fruticans* contained saponins, terpenoids, flavonoids, and phenolics. Myristicin or 4-methoxy-6-(2-propenyl)-1,3-benzodioxole is the major compound with an area of 63.45% and a similarity of 89.5%. The subfraction A (the result of fractionation of ethanol extract) reduced blood glucose level, namely 247.67 mg/dL at 30 min (98.67% compared with glibenclamide), followed by the ethanol extract as much as 203.34 mg/dL at 30 min (81.01% compared with glibenclamide). The total phenolic content and total flavonoid content of the ethanol extract of the plant were 18.0349 ± 0.4225 mg GAE/g and 2.8310 ± 0.1779 mg QE/g, respectively. The plant's ethanol extract had an antioxidant activity with IC_{50} = 417.4399 ppm.

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■ AUTHOR CONTRIBUTIONS

Conceptualization, and Methodology, Rosnani Nasution, dan Muhammad Bahi; Analysis, Muhammad Bahi, Yoon Jeon, Hadis Fadillah, dan Marianne. Investigation, and supervision, Rosnani Nasution, Hadis Fadillah, and Muhammad Bahi; writing draft preparation, Rosnani Nasution, and Hadis Fadillah; review and editing, Muhammad Bahi, and Marianne. All authors have approved this article for publication.

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