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Characteristic and bioactivities value of *Nypa fruticans* from coastal area in West Aceh District, Indonesia as a candidate antidiabetic agent

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Abstract. *Fitri Y, Yusni Y, Suryadi T, Mudatsir M. 2023. Characteristic and bioactivities value of* Nypa fruticans *from coastal area in West Aceh District, Indonesia as a candidate antidiabetic agent. Biodiversitas 24: 5260-5269. Nypa fruticans* Wurmb is a functional plant that grows on the coasts of Indonesia, produced food and has health benefits. It has been believed for generations that *Nypa fruticans* Wurmb has potential as an antidiabetic, although a complete study is needed. This research aimed to evaluate the potential of the fruit of the *Nypa fruticans* Wurmb plant as an antidiabetic based on its characteristic values and pancreatic histopathology. The fruit was evaluated for phytochemical, tannin quantity, chemical compound determination, and confirmation of bioactivity. Antidiabetic properties were evaluated for pancreatic histopathology in an animal model. *Nypa fruticans* Wurmb contains polyphenols, tannins, saponins, triterpenoids, alkaloids and flavonoids, with a tannins quantity of 11.10 mg/AT/g extract. Furthermore, it contains antioxidant, and anti-inflammatory compounds, including the dominant 2,3-Butanediol (CAS) Butane-2,3-diol, Propane-1,1-diol diacetate, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydrox, and 9,12-Octadecadienoic acid (Z,Z)- (CAS) Li. In addition, it has excellent bioactivity against nuclear receptor ligands, protease inhibitors, and enzyme inhibitors as an indicator of binding affinity with hot cells. *Nypa fruticans* Wurmb can increase beta cells, and expand the islets of Langerhans. The *Nypa fruticans* Wurmb, besides containing antioxidant and inflammatory compounds, has hight bioactivity, wich increases the frequency of beta cells and improves pancreatic islets of Langerhans repair in diabetic models.

Keywords: Antioxidant, antiinflammation, beta cells, diabetic, Langerhans, *Nypa fruticans*, pancreas

INTRODUCTION

Diabetes mellitus, an enduring metabolic disorder characterized by elevated blood glucose levels, presents a substantial worldwide health concern. The global incidence of diabetes has escalated to epidemic proportions, impacting approximately 463 million adults across the globe in 2019 (Saeedi et al. 2019). Despite the progress made in traditional treatments for diabetes, their prolonged usage frequently results in a range of adverse effects and complications. Hence, it is imperative to investigate natural sources that possess potential antidiabetic properties to facilitate the development of therapeutic interventions that are both safer and more productive (Salehi et al. 2019). The *Nypa fruticans* Wurmb is a native plant to many parts of Asia is also frequently found along the southwestern coast of Aceh, Indonesia. The plant is commonly utilized by individuals residing in the area to obtain essential nutrients (Persoon and Minter 2020).

The *Nypa fruticans* Wurmb (*Nypa*) is commonly known as the *Nypa* palm and it is primarily found in tropical areas including much of Southeast Asia (Nugroho et al. 2020). The *Nypa* exhibits a variety of distinctive attributes, rendering it a potentially good subject for investigation in the field of antidiabetic research. The substance shows a high concentration of bioactive constituents, such as polyphenols, flavonoids, saponins, tannins, and alkaloids (Rahuman et al. 2022). Extensive research has been conducted on these phytochemicals to investigate their potential as antidiabetic agents, explicitly concerning their insulin-mimetic effects, enhancement of glucose uptake, protection of pancreatic β-cells, and inhibition of glucose absorption (Ansari et al. 2022). Investigating the distinct bioactive constituents found in *Nypa fruticans* Wurmb and their respective mechanisms of action is paramount in comprehending its potential as an antidiabetic agent. Furthermore, the *Nypa* distinctive ecological niche, characterized by its ability to thrive in saline and muddy habitats, renders it susceptible to various stressors, including elevated salinity levels and limited oxygen availability (Al-Kharousi et al. 2018).

These stressors induce the synthesis of secondary metabolites in the plant, potentially contributing to its pharmacological properties. Gaining a comprehensive understanding of the adaptive mechanisms utilized by *Nypa fruticans* Wurmb in highly challenging environments could yield significant insights into its potential as an antidiabetic agent (Gul et al. 2023). In addition, using traditional

knowledge and ethnopharmacological practices about *Nypa fruticans* Wurmb can provide valuable guidance in identifying and extracting bioactive compounds. The establishment of collaborative research endeavors between indigenous communities and scientific experts has the potential to facilitate the integration of traditional knowledge and contemporary scientific methodologies, thereby facilitating the identification and exploration of innovative antidiabetic compounds (Putri et al. 2022).

The objective of this study is to conduct a comprehensive review of the distinctive attributes of *Nypa fruticans* Wurmb and assess the potential antidiabetic properties of its bioactive compounds. Furthermore, an examination will be shown of the possible synergistic effects that may arise from the interaction of various bioactive compounds and the subsequent implications this may have on the advancement of antidiabetic pharmaceuticals. The primary objective of this study is to assess the distinctive characteristics and bioactivity potential of *Nypa fruticans* Wurmb concerning its antidiabetic properties. By exploring the bioactivities linked to this particular plant species, we intend to stimulate additional investigations that may result in innovative therapeutic interventions for diabetes mellitus. This pursuit aims to enhance the overall health outcomes for individuals impacted by this widespread global epidemic.

MATERIALS AND METHODS

This research has passed research ethics No. 031/EA/FK-RSUDZA/2022 from the Faculty of Medicine and ethical clearance approval for using animals No. 114/KEPH/VII/2021 from the Faculty of Veterinary Medicine, University of Syiah Kuala, Banda Aceh, Indonesia. The research material consisted of chemical and phytochemical reagents and H and E staining materials for histopathological assessment of the *Rattus norvegicus* pancreas. In addition, this study used diabetic rats (*Rattus norvegicus*) consisting of 5 groups.

Materials

The main material in this research was *Nypa fruticans* Wurmb fruit from the coast of Kuala Bubon Village, Samatiga Sub-district, Aceh Barat District, Aceh Province, Indonesia (Figure 1). Other materials included a phytochemical screening reagent, 96% ethanol (UN1170, JT Baker), and distilled water. *Nypa fruticans* Wurmb fruit ethanol extract were also used for GC-MS analysis (Agilent Technologies 7890GC/5975MS, Japan).

Plant determination

A herbarium test was conducted in a Biology laboratory of the Faculty of Mathematics and Natural Sciences of Syiah Kuala University, Banda Aceh. The *Nypa* plant was determined to belong to the genus *Nypa* Steck with the species *Nypa fruticans* Wurmb. The botanist identified samples based on online virtual herbaria information and checked against the International Plant Names Index (IPNI) and Kewensis index. The herbarium test results data are listed in letter 426/UN 1184/TA0001/2022. Determination of this plant was essential to ensure the correctness of plant samples.

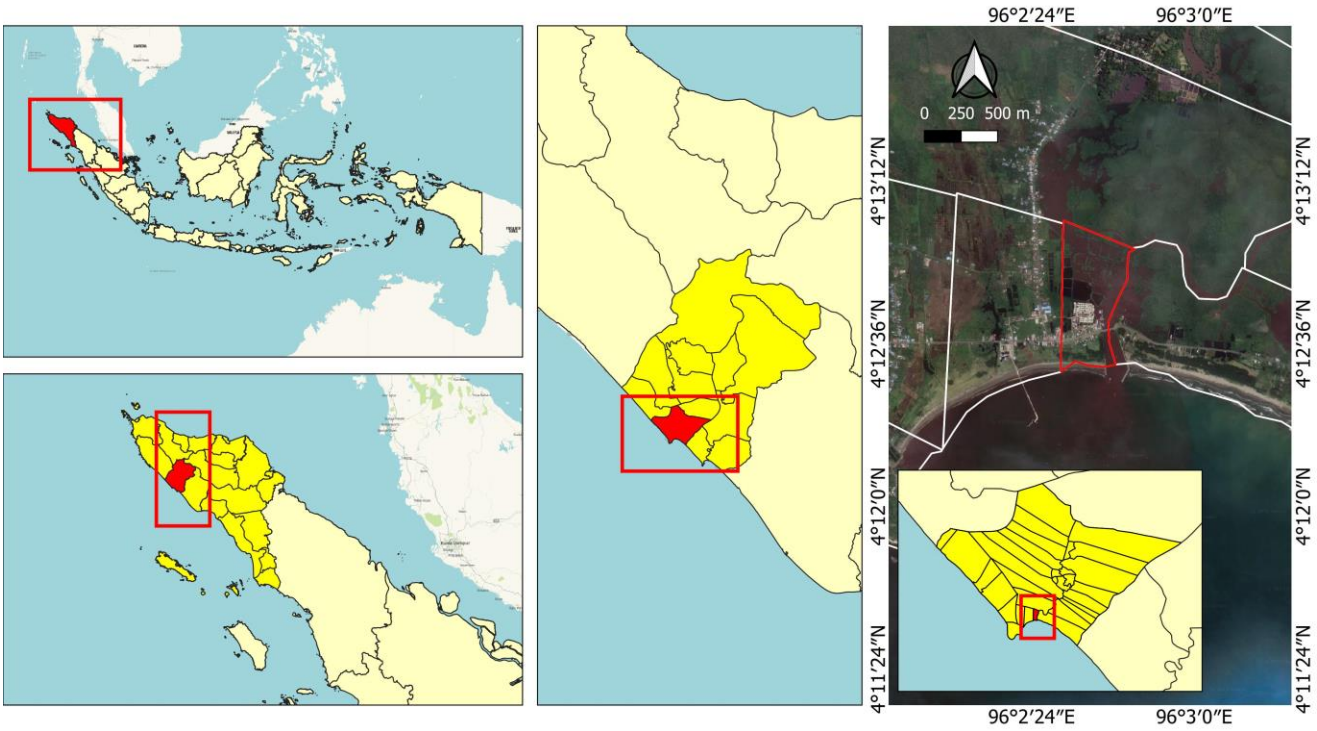

Figure 1. The sampling location in Kuala Bubon Village, Samatiga Sub-district, Aceh Barat District, Aceh Province, Indonesia

Research area and sample collection

The location/area for sampling the *Nypa* fruit is in West Aceh District, precisely in the Samatiga sub-district, which has geographic coordinates starting from 4°13'30"N and 96°02'30"E. This area has *Nypa* in areas along the coast, as well as residential areas and tourist spots. The *Nypa fruticans* Wurmb plant is a mangrove plant that belongs to the Palmae tribe and belongs to the mangrove forest plant group. *Nypa fruticans* Wurmb plants grow along the coast of West Aceh, including in the Samatiga sub-district. The samples taken were determined to be *Nypa fruticans* Wurmb. Sampling was carried out in April 2022. *Nypa fruticans* Wurmb was collected using a small boat. *Nypa fruticans* Wurmb fruit is taken by cutting the fruit hump using a machete, then collected in a boat. After the *Nypa fruticans* Wurmb hump is obtained, then the skin of the fruit is peeled by splitting it, and then the fruit is removed from the shell. After that, the fruit flesh was sliced thinly, then dried in an oven (UF 110, Germany) at 50°C for two days, then aerated at room temperature for four days so that the fruit was dry. After that, the dried fruit is blended using waring blender (8010BU,USA). The extraction process uses the maceration method with 96% ethanol solvent (UN1170, JT Baker).

Extraction and GCMS assay

A total of 2.117 kg of dry powder was extracted using ethanol as a solvent. The extraction process used the maceration method with 96% ethanol solvent (UN1170, JT Baker). The first stage begins with soaking for 72 hours and evaporating the extract. Shaking was carried out at a speed of 40 rpm, and the extraction process was carried out at a temperature of 60°C with a pressure of 80 psi using a Buchi rotary evaporator (R300, Swiss). The extract results were obtained after weighing 182 g of thick extract, which were then used in the testing process (Mawarda et al. 2020). GCMS analysis of *Nypa* ethanol extract was conducted to see the chemical compounds and the quantity contained in *Nypa* fruit extract. A total of 10 g of the test material was added to 50 mL of pro-analytical ethanol, then macerated for five days. Then 10 mL of the material was pipetted into a tube, and dried it at 60ºC for one hour. Then the remaining extract was dissolved in 200 µL of distilled water again and analyzed using the GCMS method (Agilent Technologies 7890GC/5975MS, Japan) (El-Beltagi et al. 2019).

Phytochemical assay

Flavonoids

1 mL of the extract solution was put into 4 test tubes. The first test tube was used as the control tube with nothing added while NaOH (PUDAK Scientific) was added into the second tube. Into the third tube, concentrated H_2SO_4 (Merck KgaA, Germany) was added, and concentrated Mg and HCL (Merck KGaA, Germany) powder was added into the fourth tube. The extract solution color in each of the other three non-control tubes was compared to the control tube. If there was a difference in the solution color in the three tubes, it was denoted as containing flavonoids (positive result).

Polyphenols

Three drops of the extract were put into a test tube, and then dilute $FeCl_3$ (Merck KGaA, Germany) was added into the tube. If the solution became green, orange, and red after the dilute $FeCl_3$ was added, then the solution was containing phenol (positive result).

Tannins

1 mL of the extract was put into a test tube, and 5 drops of 10% NaCl (Merck KGaA, Germany) were added. The mixture was then shaken until homogeneous and filtered to form filtrate and residue. The next step was to add 1% gelatin and 10% NaCl to the filtrate. If a white precipitate formed in the solution, then the solution was positively containing tannins.

Saponins

1 mL of the extract was put into a test tube, and 2 mL of distilled water was added. The mixture was then shaken, heated for 2-3 minutes, cooled, and shaken again. If foam formed after being shaken, then the solution was positive for containing saponins.

Triterpenoids

1 mL of the extract was put into a test tube, and 0.5 mL of acetic anhydride and 0.5 mL of $CHCl_3$ (Merck KGaA, Germany) were added. Then, 0.2 mL of concentrated H_2SO_4 (Merck KGaA, Germany) was also added, and the color change was observed. If the solution turned red, it was positive for triterpenoids.

Alkaloids

1 mL of the extract was put into a test tube, and five drops of concentrated ammonia (Merck KGaA, Germany) and 2 mL of 2 N sulfuric acid were (Merck KGaA, Germany) added. The solution was then shaken and separated. The top layer was put into three different test tubes. Into the first tube, one drop of Mayer indicator was added, and the result was denoted as positive if a white precipitate formed. Into the second tube, one drop of Dragendorff's (Merck, Germany) indicator was added. If an orange precipitate formed, the result was denoted as positive. Into the third tube, 3 mL of Wagner indicator was added. If a brown precipitate formed, the result was considered positive (Das 2014).

Determination of total tannins

A comparative solution of 1000 ppm tannic acid was prepared by dissolving 10 mg of tannic acid and methanol (Merck, Germany) until the solution volume reached 10 mL. 2.5 mL of the solution was then pipetted and diluted using methanol until it reached 25 mL and obtained a concentration of 100 ppm. Then, respectively, 1, 2,3,4, and 5 mL of the solution pipetted, mixed with methanol until the volume of 10 mL, and the concentrations of 10, 20, 30, 40, and 50 ppm were obtained. After that, 0.1 mL of the solution was then pipetted, put in a beaker, mixed with 7.5 mL distilled water with 0.1 mL Folin-Ciocâlteu (Merck KGaA, Germany) and 2 mL Na2CO3 (Merck, Germany) added. After dissolved, the solution was incubated at 37°C

for 30 minutes. The absorbance measurements were then carried out at a wavelength of 760 nm using a UV-vis spectrophotometer (Uvmini-1250, Shimadzu, Kyoto, Japan) (Ahmad et al. 2015).

Diabetic animal model

The samples in this study were a number of 25 *Rattus norvegicus* Wistar strain rats with an average weight of 200 g, an age of 2-3 months, and good health. The rats were kept in a group in the laboratory using a plastic animal cage covered with fine wire mesh. Before the treatment, the rats were acclimatized for seven days with laboratory-standardized food given without limits (*ad libitum*), in light and dark conditions, with humidity level of 60% and a temperature of 23°C. After the rats adapted to the given food for 7 days, streptozotocin (STZ) 40 mg was injected to them. The rats' fasting blood sugar levels were checked for three days, and a value of ≥126 mg/dL was denoted as suffering from diabetes mellitus (Rahma et al. 2017). The blood sugar levels were checked using the *GlucoDr* blood glucose test kit. The checking started with cleaning a rat's tail using an alcohol swab. A glut lancet was then inserted into the tip of the rat's tail until the blood came out of the rat's tail. The blood was put into a strip and read using the *GlucoDr* blood glucose test kit (Nfopia Co. Ltd, India).

Bioactivity score analysis

Molinspiration property engine software was used to calculate the effectiveness and molecular properties. The compound structures were made using ChemDraw professional v.16 © Cambridge soft software, and then the 2D designs were converted into 3D using Chem3D v.16 © Cambridge software. The bioactivity and molecular scores of *Nypa fruticans* Wrumb fruit compounds were calculated using the software mol-inspiration properties engine v. 2018.10. In the first stage, the structure of the two compounds is transferred to the mol-inspiration canvas. The computed properties button is then clicked to calculate the number of atoms, molecular weight, particles, and essential groups such as N, O, OH, and N.H. In the second stage, the effectiveness of the two test materials is calculated concerning the response to the host or infectious agent (Alberga et al. 2018; Aprilia et al. 2019).

Histopathology assessment of pancreas

The rats were euthanized and their pancreas was extracted in order to make histopathological preparations. The excised pancreases were fixed with 10% neutral buffer formalin solution, and then organ trimming was performed and the organs were put into tissue cassettes. The next step was the dehydration process using two stages of acetone (Merck, Germany) for 1.5 hours, then the two stages of cleaning with benzol for 1.5 hours. The paraffinization process was carried out with benzol + paraffin (Merck, Germany) (1:1 ratio) for 1.5 hours and paraffin for 1.5 hours. The sample is inserted into a block containing half the volume of paraffin, and the practice is placed vertically and horizontally so that the cross-section is attached to the paraffin base. After it starts to freeze, paraffin is added again until the block/mold is complete, and leave it until the paraffin hardens. The paraffin blocks were then thinly sliced 5 µm using a microtome. The resulting ribbon-shaped piece is spread over warm water at a temperature of 46°C and immediately lifted to stretch the piece so that it does not fold or lose folds due to cutting. The preparation was then removed, placed on a glass object, and dried for 18 h in an incubator of 60°C to prepare Mayer's Hematoxylin (BioMarq) and Eosin staining. Mayer Hematoxylin staining was carried out for 8 minutes and then washed in running water for 30 sec. Eosin staining was done by soaking the sample in Eosin solution for 2-3 min, then washing with running water for 30 seconds. The following process is that the preparation is dipped in 95% alcohol and absolute alcohol (I and II) 10 times. Then, the immersion was carried out gradually in absolute alcohol and xylol (Merck, Germany) I for 1 min each and then in xylol II for 2 min. The final process is closing the tissue preparation with a cover slidge (Gani et al. 2015). Histopathological observations were carried out using an electric microscope (Olympus, Tokyo, Japan).

Data analysis

Data on changes in islets of Langerhans and Beta cell frequency were analyzed by One-Way ANOVA with a significance limit of $p<0.05$.

RESULTS AND DISCUSSION

Phytochemical element and quantity of tannins of *Nypa fruticans* Wurmb fruit compared with gallic acid

Table 1 reports the results of the phytochemical screening, which showed positive results of the chemical compounds containing polyphenols, tannins, saponins, triterpenoids, alkaloids, and flavonoids. Specifically, from Table 2 the quantity of tannins in the *Nypa* fruit extract was 11.10 mg AT/g extract ($y = 0.0007x + 0.0149$ with an R2 value=0.98). This suggests that *Nypa* fruit can potentially prevent infection and increase glucose metabolism. Figure 2 shows the chromatogram of the *Nypa* fruit extract. The gas chromatography analysis was in tandem with a mass spectrometer to obtain the relative mass of each compound. The data from the instrument was compared with the NIST compound database. Twenty-two compounds of *Nypa* fruit from classes were detected and tabulated in Table 3. The six major components that predict the antioxidant compound are numbers 10, 16, 20, and 32.

***Nypa fruticans* Wurmb fruit extract GC-MS analysis**

Table 4 shows 4 (No. 30, 31, 32, and 33) chemical compounds from the *Nypa* fruit that have nuclear receptor ligands, protease inhibitors, and enzyme inhibitors above the minimum value (-1>). In particular, these four compounds had higher protein coupled receptors and ion channel modulator values than other bioactivity variables. This value indicates the kinetic properties of chemical compounds when they adapt to cells and protect cells from the threat of infection and the effects of ROS responses. Apart from that, an analysis of the structure of the chemical

compounds contained in the *Nypa* fruit extract was also carried out, which can be seen in Figure 3.

Diabetic assessment of pancreas histopathology after treatment with *Nypa fruticans* fruit

Table 5 reports changes in the islets of Langerhans and the population of beta cells in the islets of Langerhans in a diabetic animal model after being treated with *Nypa* fruit extract and an antidiabetic drug (metformin). The 500 mg *Nypa* fruit extract +500 mg metformin group repaired the islets of Langerhans and increased the number of beta cells close to that of the normal group. Meanwhile, the 500 mg/Kg BW extract group and the positive control group (500 mg Metformin) were close to the normal group values but still within the standard threshold. In general, all treatment groups could repair the islets of Langerhans, which were damaged after being induced with diabetic factor (STZ).

Table 1. Phytochemical element of *Nypa fruticans* fruit

Phytochemical elements	Results
Polyphenol	+
Tannins	+
Quinone	-
Saponins	+
Triterpenoids	+
Steroids	-
Alkaloids	+
Flavonoids	+

Table 2. Quantity of tannins of *Nypa fruticans* Wurmb fruit compared gallic acid

Tannin quantity (mg AT/g extract)			
OD	**SD**	**Quantity**	**Calibration Curva**
0.023	0.67	11.10	$R^2 = 0.98$

Table 3. GC-MS evaluation of chemical compound of *Nypa fruticans* fruit extract

Retention times	Chemical compounds	Area %
3.025	Formic acid (CAS) Bilorin	0.94
3.095	Hydrazine, methyl- (CAS) Methylhydrazin	0.69
3.555	2-Propanone, 1-hydroxy- (CAS) Acetol	0.70
3.820	Ammonium acetate	9.70
3.950	Acetic acid, hydrazide (CAS) 374	2.95
5.212	Acetic acid, methyl ester	0.94
5.527	Propanoic acid, 2-oxo-, methyl ester (CAS)	3.50
5.650	1,4-Dioxadiene	4.27
5.755	Pyrrolidine-.Alpha., Alpha.ALP	1.70
5.999	2,3-Butanediol (CAS) Butane-2,3-diol	12.55
6.623	2-Furanmethanol	2.25
7.588	2(5H)-Furanone	1.07
7.877	1,2-Cyclopentanedione	2.01
8.611	2,4-Dihydroxy-2,5-dimethyl-3(2H)- furan-3	1.08
9.652	Benzeneacetaldehyde (CAS) Hyacinthin	1.50
9.760	Propane-1,1-diol diacetate	3.34
9.835	Butanoic acid, 3-hydroxy-	1.58
10.487	2,3-Dihydro-5-hydroxy-6-methyl-4H- pyran	0.65
11.570	.alpha.-Hydroxyisocaproic acid	1.19
11.866	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydrox	12.89
12.035	Glycerin	3.55
12.434	Catechol	1.28
12.689	Dianhydromannitol	0.64
12.936	5-Hydroxymethylfurfural	7.08
13.959	Hydroquinone	0.69
16.958	Dodecanoic acid	0.47
18.485	Hydrazinecarboxamide, 2-(2- methylcycloh	1.61
18.822	Bicyclo [3.3.1]Non-1-Methoxy-3-O	1.52
20.341	Caffeine	0.29
21.110	9-Hexadecenoic acid	1.11
21.383	n-Hexadecanoic acid	9.03
23.002	9,12-Octadecadienoic acid (Z,Z)- (CAS) Li	6.66
23.180	Octadecanoic acid	0.58

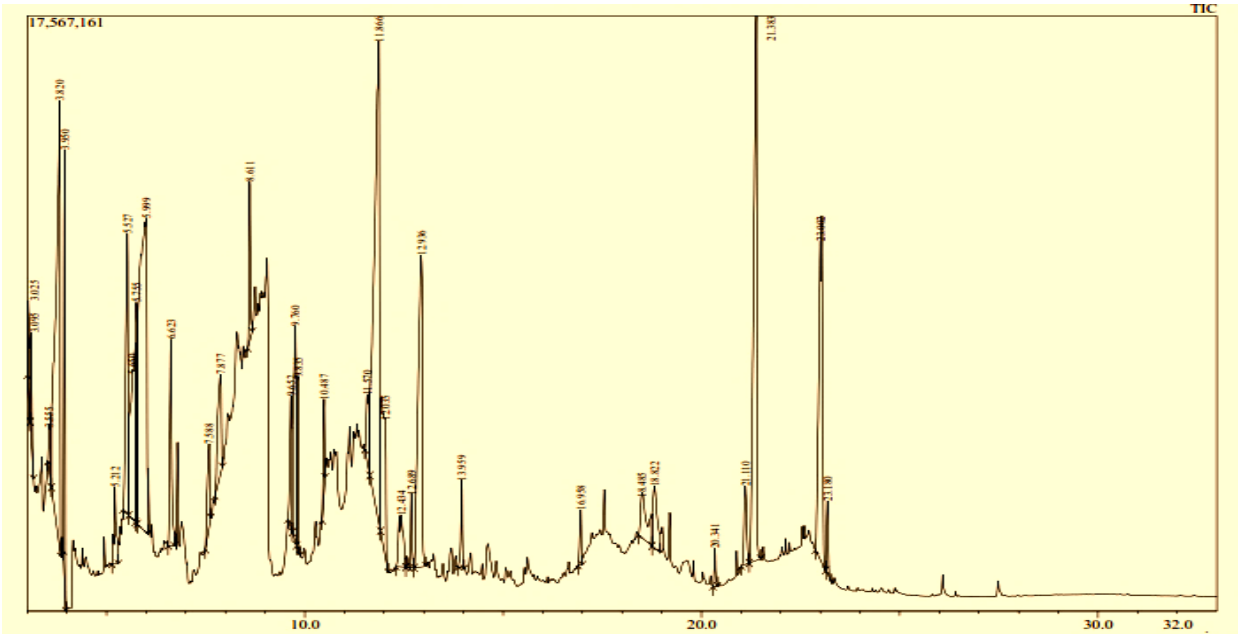

Figure 2. G.C. Spectrum of *Nypa fruticans* fruit leaves extract. The peaks started at 3.025 min of retention until 23.180 min

Table 4. Bioactivity score of the chemical compound of *Nypa fruticans* fruit

Compound	Bioactivity					
	GPCR Ligand	ICM	Kinase Inhibitor	NRL	Protease inhibitor	Enzyme inhibitor
Formic acid	-3.77	-3.76	-3.84	-3.73	-3.73	-3.33
Methylhydrazin	-3.97	-4.06	-3.75	-4.04	-3.68	-3.72
2-Propanone, 1-hydroxy	-3.81	-3.77	-3.96	-3.64	-3.68	-3.57
Ammonium acetate	-3.80	-3.77	-3.82	-3.79	-3.78	-3.77
Acetic acid, hydrazide	-3.94	-4.10	-3.85	-4.10	-3.80	-3.75
Acetic acid, methyl ester	-3.77	-3.70	-3.88	-3.69	-3.70	-3.69
Propanoic acid, 2-oxo-, methyl ester	-3.71	-3.48	-3.85	-3.59	-3.44	-3.41
1,4-Dioxadiene	-3.69	-3.66	-3.65	-3.70	-3.67	-3.63
Pyrrolidine-.Alpha.	-3.38	-3.05	-3.46	-3.88	-3.20	-3.40
2,3-Butanediol	-3.67	-3.63	-3.69	-3.61	-3.63	-3.49
2-Furanmethanol	-2.05	-2.17	-2.96	-2.48	-2.95	-1.89
2(5H)-Furanone	-3.27	-3.70	-3.83	-3.71	-3.67	-3.55
1,2-Cyclopentanedione	-3.56	-3.29	-3.69	-3.49	-3.40	-3.14
2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	-1.60	-0.64	-1.98	-1.29	-1.54	-0.52
Benzeneacetaldehyde	-2.16	-1.41	-2.39	-2.18	-1.82	-1.57
Propane-1,1-diol diacetate	-0.79	-0.35	-1.26	-1.10	-0.72	-0.06
Butanoic acid, 3-hydroxy	-3.15	-2.89	-3.69	-2.92	-2.82	-2.48
2,3-Dihydro-5-hydroxy-6-methyl-4H-pyran	-2.92	-2.17	-3.54	-2.83	-2.56	-1.76
.alpha.-Hydroxyisocaproic acid	-1.80	-1.29	-2.69	-1.59	-1.50	-1.24
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy	-2.65	-2.01	-3.35	-2.82	-2.44	-1.86
Glycerin	-3.37	-3.37	-3.54	-3.48	-3.52	-3.17
Catechol	-3.04	-2.51	-3.10	-2.98	-3.24	-2.67
Dianhydromannitol	-0.89	-0.54	-0.97	-1.07	-0.58	0.04
5-Hydroxymethylfurfural	-2.71	-2.19	-2.92	-2.73	-3.21	-2.24
Hydroquinone	-3.02	-2.48	-3.07	-2.84	-3.20	-2.66
Dodecanoic acid	-0.27	-0.04	-0.75	-0.24	-0.36	0.04
Hydrazinecarboxamide, 2-(2-methylcycloh	-0.70	-0.69	-1.04	-0.83	-0.35	-0.22
Bicyclo[3.3.1]Non-1-Methoxy-3-One	-1.23	-0.91	-2.68	-0.38	-1.07	-0.21
Caffeine	-0.53	-0.98	-1.07	-2.10	-1.23	-0.22
9-Hexadecenoic acid	0.08	0.08	-0.35	0.14	-0.04	0.26
n-Hexadecanoic acid	0.02	0.06	-0.33	0.08	-0.04	0.18
9,12-Octadecadienoic acid (Z,Z)-	0.29	0.17	-0.16	0.31	0.12	0.38
Octadecanoic acid	0.11	0.05	-0.20	0.17	0.06	0.20

Note: ICM: Ion channel modulator, NRL: Nuclear receptor ligand

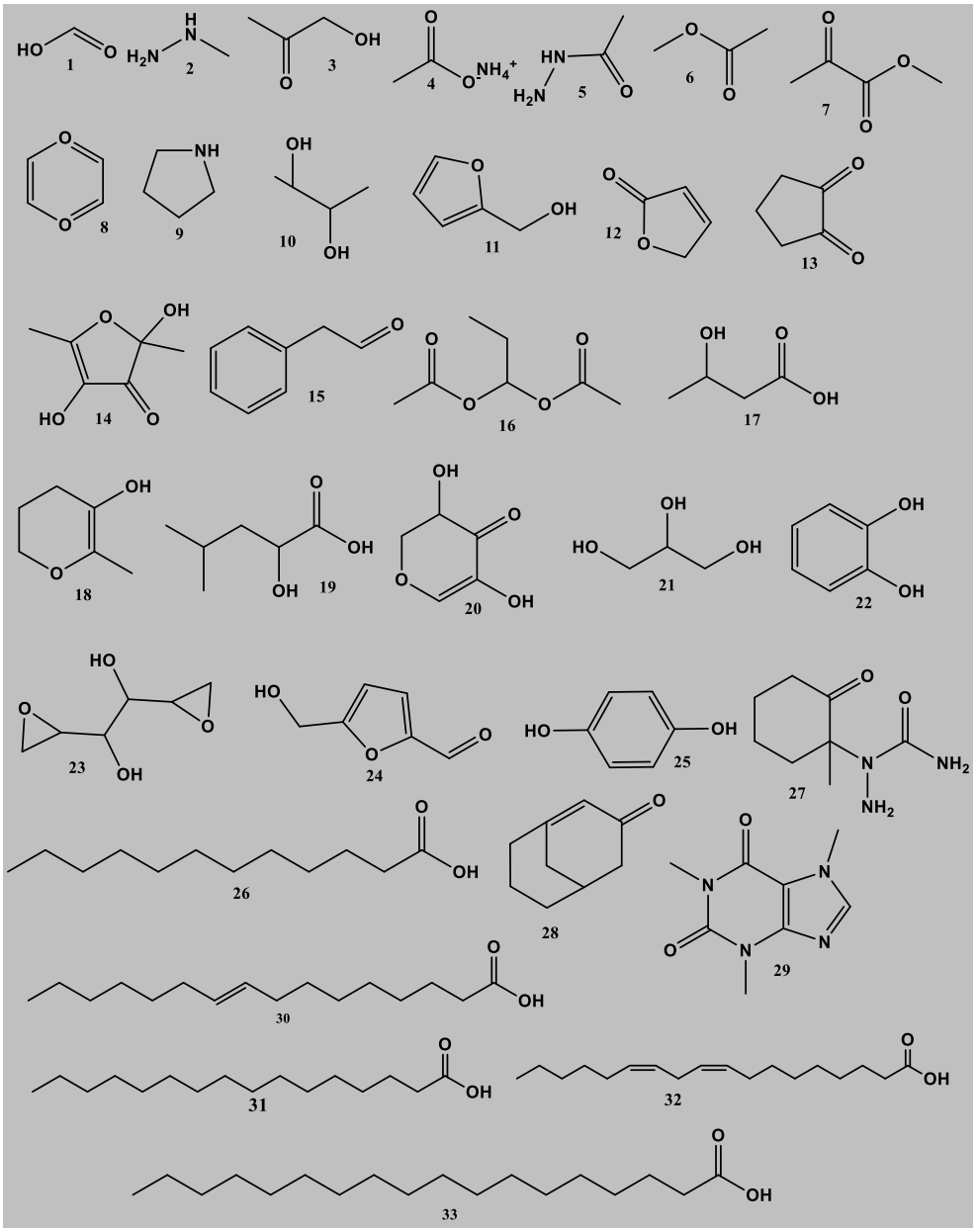

Figure 3. The structure of the chemical compound *Nypa fruticans* fruit with O and O.H. chains is owned by each chemical compound

Table 5. Diabetic assessment of pancreas histopathology after treatment with *Nypa fruticans* fruit

Nypa fruit	N	Diabetic assessment of pancreas histopathology			
		Langerhans (mm)		Beta cells (%)	
		Area	SD	Amount	SD
500 mg	5	7.848	2.565	5.3	1.2
500 mg+Metformin 500 mg)	5	8.811	1.728	6.2	0.5
C+ (Metformin 500 mg)	5	8.354	1.083	5.9	1.4
Negative Control	5	3.512	1.165	3.0	0.7
Normal	5	9.347	9.405	6.2	1.1
**p-value*		0.041		0.015	

Note: *One Way ANOVA

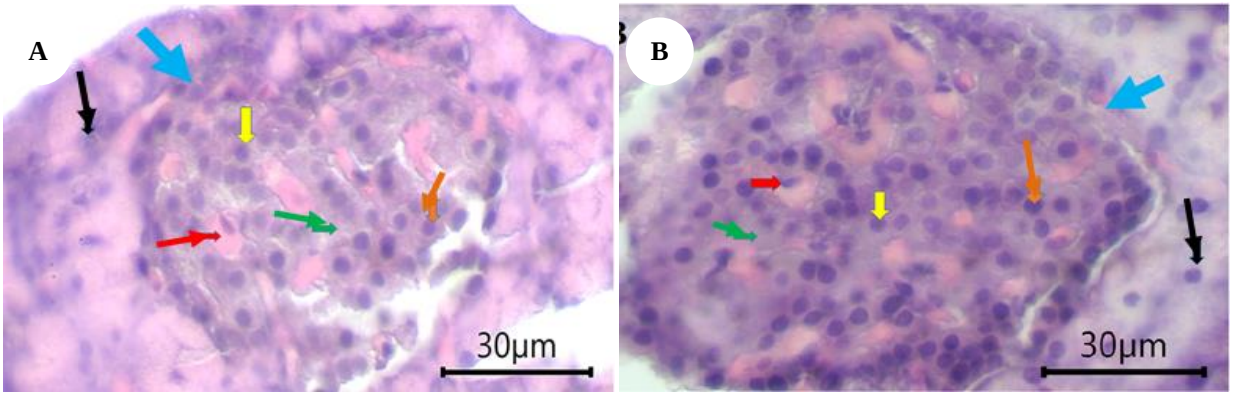

Figure 4. Pancreatic histopathology from *Rattus norvegicus* with diabetes. A. Representative of the negative control group, B. Representative of the *Nypa* fruit extract treatment group. Blue arrows: Islands of Langerhans, yellow arrows: beta cells, orange arrows: alpha cells, red arrows: blood capillaries with hematoma, green arrows: cytoplasm, black arrows: acinar cells. H and H staining at 400x magnification

Figure 4 reports the histopathological profile of the pancreas from a diabetic model treated with *Nypa* fruit extract. Figure 4.B shows the presence and expansion of the islet of Langerhans and the different alpha cell populations after being treated with *Nypa* fruit extract as indicators of pancreatic healing/repair. Figure 4.A shows a reduction in the islets of Langerhans, and the population of beta and alpha cells shows a drastic decrease based on the image profile.

Discussion

Nypa fruit parts of the *Nypa fruticans* Wurmb plant have been used to treat various health conditions, including diabetes (Sendang 2019). However, it's important to note that traditional uses do not always translate to scientifically proven therapeutic effects. While some studies have investigated the potential antidiabetic properties of *Nypa fruticans* Wurmb extracts in animal models, human clinical trials and robust scientific evidence supporting its effectiveness as an antidiabetic treatment are lacking at this time (Budiwati and Kriswiyanti 2014).

Nypa fruticans Wurmb contains polyphenol compounds, tannins, saponins, triterpenoids, alkaloids, and flavonoids, with a tannin quantity of 11.10 mg/AT/g extract. These results indicate that *Nypa fruticans* Wurmb fruit has potential as an antioxidant and anti-inflammatory, which is clarified by the findings in Table 2. The secondary metabolites contained in the nipa palm fruit extract are also very beneficial to one's health. Polyphenols serve as antioxidants, tannins are antihyperglycemic and function in cell degeneration, alkaloids are antihyperglycemic and work against cell degeneration, flavonoids serve as antioxidants and function in ß cell degeneration, and phytosterols serve as anticholesterol agents (Prasad et al. 2013; Syarpin et al. 2023).

As an antidiabetic, tannins are antibacterial and interfere with protein transport in the inner layer of cells. Tannins are reported to work to increase glucose and fat metabolism. Besides having antioxidant activity, tannins also play a role in reducing blood sugar levels through increasing glycogenesis metabolism (Alam et al. 2022). Tannins also reduce the absorption of food essences through their function as an astringent by shrinking the smooth epithelial membrane. This causes blood sugar absorption to slow down so that the increase in blood sugar levels is not too high (Laklaeng and Kwanhian 2020).

The phytochemical compounds possessed by *Nypa fruticans* Wurmb fruit, apart from working as antioxidants, anti-inflammatories, and anti-cholesterol agents, are also immunomodulators to prevent chronic diseases and reduce the pathological impact on various diseases (Yahfoufi et al. 2018). Flavonoids are reported to neutralize free radicals so that the effects of damage to cells and body tissues can be minimized. The antidiabetic activity of flavonoids acts on pathways by regenerating cells in the islets of Langerhans. Langerhans cell repair will increase the amount of insulin so that blood glucose can enter the cells and decrease blood glucose levels (Al-Ishaq et al. 2019). Triterpenoid compounds are found in various plant kingdoms, and currently, the biological and pharmacological properties of these compounds are currently the focus of research and drug development. Triterpenoid components in plants have been used as traditional antidiabetic medicine in many countries. Natural triterpenoids have pharmacological activity as antidiabetic agents; based on the results of an article review, it was found that triterpenoids are emerging candidates to be developed for the treatment of diabetes mellitus (Lyu et al. 2016).

As shown by the chromatogram peaks from the GCMS analysis in Figure 2, the *Nypa* fruit extract contained 33 bioactive compounds, 6 of which had percentages exceeding 5% while the remaining 27 were below 5%. The main compounds of the *Nypa* fruit extract are: ammonium acetate, 2,3-butanediol (CAS) butane-2,3-diol, 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy, 5-hydroxymethylfurfural, n-hexadecanoic acid, and 9,12-octadecadienoic acid (Z,Z)-(CAS) Li. Linoleic acid or 9,12-octadecadienoic acid (Z,Z)-(CAS) Li has anti-inflammatory, hepatoprotective, carcinopreventive, antihyperglycemic, antioxidant, and hypocholesterolemic effects (Salem et al. 2016; Aziz et al.

2020; Zaky et al. 2022). 9,12-Octadecadienoic acid (Z,Z) also has the potential to protect the pancreas from damage (Osman and Hussein 2014). The study results showed that long-chain monounsaturated fatty acids and a diet rich in linoleic acid could stimulate and increase the release of GLP-1 in murine models and human L-cells and increase blood insulin levels. Linoleic acid regulates insulin sensitivity and influences glucose homeostasis through gene regulation, lipid metabolism, and adipocyte development (Paschoal et al. 2020).

The data in Table 5 show that *Nypa fruticans* Wurmb fruit can increase the repair of islets of Langerhans, and increase beta cells, as an indicator of the improved function of insulin produced by beta cells. From a theoretical perspective, flavonoids or antioxidant compound that *Nypa fruticans* Wurmb have can increase GLUT translocation activity, glucose uptake by tissues, and liver enzyme activity, causing a decrease in apoptosis and inhibition of tyrosine kinase to prevent an increase in diabetes mellitus (Sun et al. 2023). This property aligns with the data in Table 4, where several antioxidant compounds possessed by *Nypa fruticans* Wurmb have bioactivity with receptor kinases, ion channel modulators and nuclear receptor ligands, protease inhibitors, and enzyme inhibitors.

The pharmacological effects of flavonoid compounds have long been known to act as antidiabetic agents by increasing insulin secretion and reducing apoptosis, pancreatic cell proliferation, improving hyperglycemia, and reducing insulin resistance (Vinayagam and Xu 2015). This effect was proven based on research conducted on experimental animals, which showed that the high content of flavonoids in *Pilea microphylla* extract has antidiabetic properties by inhibiting DPP-IV and increasing antioxidant levels in diabetes rats (Bansal et al. 2012).

Figure 4 shows that *Nypa fruticans* Wurmb fruit can improve pancreatic function, characterized by enhanced islets of Langerhans and increased beta cells. In addition, antidiabetic activity occurs by inhibiting the activity of α-glucosidase and α-amylase enzymes, inhibiting DPP-IV, and increasing GLUT-1 and GLUT-4 translocation. The triterpenoid class stimulates insulin receptor substrate-1 (IR-1), inhibiting α-glucosidase activity, increasing GLUT-4 and glucose absorption (Shehadeh et al. 2021). In addition, triterpenoids can significantly reduce the formation of free radicals (ROS) in cells by improving various antioxidant enzymes such as superoxide dismutase 1 (SOD1), catalase, glutathione peroxidase-1 (GPx-1) and thioredoxin reductase (TrXR) either in vivo or in vitro (Song et al. 2022).

Based on the findings of this study, we can report that *Nypa* fruit has the possibility to be developed as an antidiabetic, where its antioxidants can protect beta cells by reducing cell damage due to oxidative stress. In addition, as an anti-inflammatory, it has the opportunity to reduce inflammation and increase insulin sensitivity (Nugroho et al. 2020). Furthermore, the active compound found in *Nypa* fruit might potentially protect existing beta cells from damage and stimulate the regeneration of new beta cells. It could help improve insulin production and secretion in individuals with diabetes (Saad et al. 2017). Also, plant extracts may help regulate blood glucose levels by influencing glucose uptake in cells or inhibiting enzymes that break down carbohydrates, leading to better glycemic control and the potential to inhibit AGE (Advanced Glycation End Products) formation, thereby reducing the impact of AGEs on diabetic pathology (Unuofin and Lebelo 2020; Golovinskaia and Wang 2023). Further study on the efficacy of these phytochemical contents in the medical and pharmaceutical fields is needed.

In conclusion, the *Nypa fruticans* Wurmb fruit, apart from containing antioxidant and inflammatory compounds, also has good bioactivity and can improve the repair of the islets of Langerhans in the pancreas, and increase the frequency of beta cells in animal diabetic models.

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