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POSTPRANDIAL GLYCEMIC REGULATING EFFECT OF ENSETE GLAUCUM SEED EXTRACT

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Summary

Postprandial Glycemic Regulating Effect of *Ensete glaucum* Seed Extract

Recently, plant-based products have been given much attention as a cure for type 2 diabetes. Acknowledging the issue, the current study was aimed at evaluating the potential of *Ensete glaucum* seed extracts in postprandial glycemia management. *E. glaucum* seed extracts were examined in the *in vitro* α -amylase and α -glucosidase inhibitory assay to detect the potential extract. After that, an oral glucose tolerance test on mice was applied for the *in vivo* study to test the effect of the potential extract in postprandial glycemia management by measuring plasma glucose levels. The results illustrated that *E. glaucum* seed extracts had the ability to inhibit these enzymes, in which 96% ethanol extract by maceration with an IC₅₀ value of 0.496 mg/mL in α -amylase inhibitory activity and 96% ethanol extract from hot extraction stood out from other extracts with an IC₅₀ value of 1.511 μ g/mL in α -glucosidase inhibitory activity. The hot-extracted 96% ethanol extract also had antioxidant activity *via* DPPH and ABTS inhibition with IC₅₀ values of 10.98 μ g/mL and 12.03 μ g/mL, respectively. Seven-day administration of the hot-extracted 96% ethanol extract (50 mg/kg b. w., *p. o.*) significantly reduced the plasma glucose level in mouse oral glucose tolerance test. These preliminary findings suggested that *E. glaucum* seeds had potent anti-hyperglycemic activity, and contributed more in supporting diabetes regimes. Further studies need to be conducted to clarify the effect of *E. glaucum* seeds on diabetes.

Keywords: *Ensete glaucum* seeds, Ethanol extract, α -Amylase, α -Glucosidase, Oral glucose tolerance test, Postprandial glycemia.

1. Introduction

Type 2 diabetes, which is also previously known as adult-onset diabetes, non-insulin-dependent diabetes, accounts for roughly 90 to 95% of those living with diabetes. Half of people suffering from this chronic disease do not know their status [1]. In general, this chronic disease medication is not needed for insulin treatment

throughout a patient's lifetime; however, its consequences relate to the risk of developing macrovascular and microvascular complications [2]. In Vietnam, one in every 20 people is diagnosed with diabetes. By the end of 2015, the number of people with diabetes is up to 63 021 cases, about 5.6% of people 20 to 75 years [3]. In 2035, the incidence of diabetes and prediabetes

would be projected to be 7% and 15.7%, respectively. As Vietnam is a developing country, its incidence of diabetes has been on an alarming increase, nearly doubling in the past 10 years [4].

One of the treatments for type 2 diabetes is to control postprandial blood glucose excursion through the inhibition of α -amylase and α -glucosidase activity that mainly involves carbohydrate hydrolysis and intestine glucose absorption [5]. In the marketplace, acarbose is a widely used and well-known drug for the above-mentioned mechanism [6]. The use of medicinal herbs, which are naturally extracted from plants, has become one of the users' priorities and preferences. According to the report of the World Health Organization (WHO), medicinal herbs provide the needs of approximately 80% of the world's population. Plant materials provide a cheap source of medicine and bring many benefits in reducing side effects, lowering costs, and strengthening relief and assurance for consumers. In practice, many phytochemicals and bioactivities have been recorded in banana seeds from several species such as *Musa balbisiana*, *Ensete superbum*, and *Musa acuminata*. They are proven to have various pharmacological effects such as antioxidant activity, antidiabetic activity, antimicrobial activity, anticancer effect, wound healing attribute, and effects on atherosclerosis [7],[8],[9].

In Vietnam, there is a banana species that is rumored to have medicinal properties, especially the seeds of this species would be utilized to make many remedies for diabetes, kidney stones, or diuretic treatments. The name of this species is *Ensete glaucum*, also known as snow banana or "Chuoi co don" classified in the family Musaceae [10]. Compared to other species, *E. glaucum* poses a unique characteristic in which all the nutrients from dead plants will concentrate on its seldom fruit in one bunch. In this way, their seeds which are extraordinary fruit applied traditionally for food and medicinal purposes. In some cases, *E. glaucum* seeds are used as diabetic remedies in the form of decoction or alcohol soaked from raw seeds. For these reasons, this study was to initially evaluate *in vitro* inhibitory activity of α -amylase and α -glucosidase of crude extracts of the *E. glaucum* seeds and follow-up to *in vivo* activity in animal

models to confirm more strongly their effect in postprandial glycemia management.

2. Materials and methods

2.1. Plant materials and extracts

Seed materials derived from *Ensete glaucum* (Roxb.) Cheesman species, which were collected in April 2020 from Bac Ai District, Ninh Thuan Province, Vietnam. These dried seeds were ground to a fine powder (2 mm sieve) for extraction. Crude extracts included 96% ethanol extract and 45% ethanol extract by maceration method, 96% ethanol extract and 45% ethanol extract by hot extraction, and aqueous extract by decoction method. These extracts were derived from our previous study [11].

2.2. α -Amylase inhibitory assay

This assay was carried out according to the previously described with some calibrations [12]. Briefly, 250 μ L of each extract at different concentrations or acarbose as a positive control was incubated with 250 μ L α -amylase (1.25 U/mL, from malt, Himedia, India) in phosphate buffer at 37°C for 15 min. After that, 250 μ L of starch 1% as a substrate was added and incubated at 37°C for more than 20 min. Then, 500 μ L of the DNSA color reagent (Jinhua, China) was pipetted and boiled for 5 min to stop the reaction. Finally, the mixture was cooled to room temperature and absorbance was recorded at a wavelength of 540 nm. The measurements were performed in triplicate.

2.3. α -Glucosidase inhibitory assay

This test was conducted as described previously [12]. Briefly, 60 μ L of each extract at different concentrations or acarbose as a positive control was incubated with 50 μ L of α -glucosidase (0.2 U/mL, from *Saccharomyces cerevisiae*, Sigma) in 1 M phosphate buffer (pH 6.8) at 37°C for 10 min. Then, 50 μ L of 5 mM pNPG (Sigma) was added to this mixture as a substrate and incubation was continuously kept at 37°C for 20 min. The absorbance was measured at a wavelength of 405 nm. The measurements were performed in triplicate.

Percentage of enzyme inhibition was calculated by this expression: $I(\%) = [(A_c - A_{0c}) - (A_t - A_{0t})] / (A_c - A_{0c}) \times 100$. Where A_c , A_{0c} , A_t , A_{0t} are the absorbance of the samples with enzyme but without test extract, without enzyme and test

extract, with enzyme and test extract, and with extract but without enzyme, respectively.

2.4. *In vitro* antioxidant assays

DPPH radical scavenging and ABTS radical cation decolorization assays were used to evaluate the antioxidant activity of *E. glaucum* seed extract. These assays were conducted and the results were analyzed as described previously [11].

2.5. Experimental animals

Swiss albino mice of both sexes (about 5-6 weeks old) obtained from the Institute of Vaccines and Medical Biologicals in Nha Trang City were used for this study. The mice were kept in the animal house at a temperature of 25°C, humidity at 50 - 65%, and on light-dark cycles (12 hours shifts) of the Department of Pharmacology-Biochemistry, Research Center of Ginseng and Medicinal Materials. The animals were acclimatized for one week to their feeds and water which they had access to *ad libitum*. All the animal studies were carried out according to the guidelines of the "Guidelines for the Care and Use of Laboratory Animals" and this research work and protocols were approved by the institutional animal ethical committee of University of Medicine and Pharmacy at Ho Chi Minh City, Ho Chi Minh City, Viet Nam (Ref: 591/GCN-HĐĐNCTĐV).

2.6. Acute oral toxicity test

Mice were fasted for 12 hours before extract administration. The study was carried out in two phases. In the preliminary phase, six mice (3 males and 3 females) were administered the extract orally at the highest concentration can be delivered into the stomach by a gavage needle (50 mL/kg volume). These animals were observed for signs of toxicity and mortality for 72 hours. The results of this phase informed the choice of doses for the determination phase. In the case of D_{max} determination, the number of mice is increased (50% male, 50% female). These animals were observed for signs of toxicity and mortality for 72 hours. Mice were dissected to observe the macroscopic and microscopic organs (if there was death). If the dose D_{max} is determined, the relatively safe dose (D_s) used in pharmacological experiments may be $1/5D_{max}$ or greater than $1/5D_{max}$ [13].

2.7. Oral glucose tolerance test in mice

After acclimatization of the mice, male mice were distributed into four groups with the average plasma glucose level and weights among the groups being nearly the same as follows: Group 1 (control group) received standard mouse feeds and 10 mL/kg of distilled water; Group 2 received *E. glaucum* seed extract at 50 mg/kg/day; Group 3 received *E. glaucum* seed extract at 100 mg/kg/day; Group 4 received *E. glaucum* seed extract at 200 mg/kg/day; Group 5 (reference drug) received glibenclamide at 5 mg/kg. The extracts were administered single dose or repeated daily doses throughout the duration of the experiment (7 days) while glibenclamide used only a single dose on the day of the test.

Overnight-fasting normal mice were taken tail blood samples to determine plasma glucose levels as initial levels. After that, mouse groups received corresponding samples. In this study, glucose solution (2 g/kg, *p.o.*) was administered 60 min after vehicle/ *E. glaucum* seed extract/ glibenclamide administration [14],[15]. Blood samples were collected at 30, 60 and 120 min after glucose overload. The plasma glucose level was estimated using the glucose kit (Erba, Germany) according to the glucose oxidase method.

Bloods (10 μ L) were taken out and put into 10 μ L EDTA 2%, then centrifuged at 5000 rpm/5 min to obtain 5 μ L plasma which was incubated gently with 0.25 mL test indicator agent at 25°C for 10 minutes. The glucose concentration was calculated through absorbance of the reactive mixture at 500 nm (Glucose standard 100 mg/mL). The level of glucose is estimated by the following formula: Level of glucose (mg/dL) = $(A_s - A_b)/(A_0 - A_b) \times 100 \times \text{dilution}$. Where A_s , A_b , and A_0 are absorbance values of test, blank, and standard samples, respectively.

The percent lowering of average plasma glucose levels was calculated according to the formula: Glucose reduction (%) = $(1 - C_{\text{test}}/C_{\text{control}}) \times 100$, where C_{test} represents the average plasma glucose concentration in glibenclamide or *E. glaucum* seed extract administered mice (Groups 2–5), and C_{control}

represents in the control mice (Group 1), respectively.

The area under the curve (AUC) was calculated for each group using the equation [16]: $AUC (mg \cdot dL^{-1} \cdot min) = [(C_0 + C_{30}) \div 2] \times (t_{30} - t_0) + [(C_{30} + C_{60}) \div 2] \times (t_{60} - t_{30}) + [(C_{60} + C_{120}) \div 2] \times (t_{120} - t_{60})$. Where C_0 , C_{30} , C_{60} , C_{120} are plasma glucose concentrations at 0 min (t_0), 30 min (t_{30}), 60 min (t_{60}), and 120 min (t_{120}), respectively.

2.8. Statistical analysis

Data were expressed in terms of Mean $\pm$ Standard deviation. The IC_{50} value was determined from the curve of log dose-response (X-axis) and percentage of inhibition (Y-axis) calculated from

the absorbance values. From this sigmoidal curve, IC_{50} (X value) was calculated by setting the Y value as 50% using GraphPad Prism software (version 8.0.1, Inc., La Jolla, CA, USA). The data were checked for normality by the Anderson-Darling test and analyzed by the One-way analysis of variance (ANOVA) followed by Tukey's test for homogeneity of variance and Dunnett's test for heterogeneity of variance ($p < 0.05$ is statistically significant).

3. Results

3.1. α -Amylase and α -glucosidase inhibitory activities of *E. glaucum* seed extracts

Table 1. α -Amylase inhibitory activity of *E. glaucum* seed extracts

Extract	% Inhibition					IC_{50} (mg/mL)
	0.125	0.25	0.50	0.75	1.00	
E1	17.58 $\pm$ 3.39	27.93 $\pm$ 1.54	50.25 $\pm$ 1.69	60.82 $\pm$ 1.75	80.87 $\pm$ 4.47	0.496
E2	11.23 $\pm$ 2.16	19.91 $\pm$ 1.26	47.81 $\pm$ 3.88	61.73 $\pm$ 6.48	79.65 $\pm$ 5.49	0.546
E3	20.77 $\pm$ 2.04	28.19 $\pm$ 1.49	43.87 $\pm$ 3.05	62.23 $\pm$ 1.81	87.00 $\pm$ 5.60	0.538
E4	13.98 $\pm$ 3.10	19.04 $\pm$ 3.65	37.01 $\pm$ 5.69	56.28 $\pm$ 1.04	81.82 $\pm$ 1.88	0.664
E5	12.66 $\pm$ 2.74	19.17 $\pm$ 1.82	36.19 $\pm$ 1.02	52.88 $\pm$ 4.14	80.13 $\pm$ 1.84	0.682

E1 and E3: 96% ethanol and 45% ethanol extracts by maceration, E2 and E4: 96% ethanol and 45% ethanol extracts by hot extraction, E5: aqueous extract by decoction. Mean $\pm$ SD, $n = 3$.

Table 2. α -Glucosidase inhibitory activity of *E. glaucum* seed extracts

Extract	% Inhibition				IC_{50} (μ g/mL)
	0.59	1.17	2.34	4.69	
E1	10.31 $\pm$ 1.55	26.19 $\pm$ 4.54	54.27 $\pm$ 3.32	91.94 $\pm$ 0.43	2.093
E2	11.48 $\pm$ 3.40	37.39 $\pm$ 2.28	71.61 $\pm$ 3.53	94.22 $\pm$ 2.82	1.511
E4	19.76 $\pm$ 2.18	29.84 $\pm$ 4.65	47.57 $\pm$ 4.41	74.87 $\pm$ 1.45	2.943
E3	18.70 $\pm$ 4.88	43.06 $\pm$ 6.05	65.37 $\pm$ 2.94	93.58 $\pm$ 1.24	2.350
E5	29.60 $\pm$ 6.64	54.90 $\pm$ 3.23	63.94 $\pm$ 12.51	90.70 $\pm$ 0.25	9.289

E1 and E3: 96% ethanol and 45% ethanol extracts by maceration, E2 and E4: 96% ethanol and 45% ethanol extracts by hot extraction, E5: aqueous extract by decoction. Mean $\pm$ SD, $n = 3$.

In order to find out *E. glaucum* seed extract with potential effect towards antidiabetic, α -amylase and α -glucosidase inhibitory activities of crude extracts from *E. glaucum* seeds were evaluated. The results illustrated that all crude extracts from *E. glaucum* seeds were able to inhibit α -amylase and α -glucosidase activities. The percentage of inhibition from five extracts was upward proportional to the concentrations

and about 80% at 1 mg/mL. At 500 μ g/mL, 96% ethanol extract by maceration expressed over 50% more inhibitory activity than the others (Table 1). Similarly, the crude extracts from *E. glaucum* seeds demonstrated their ability in α -glucosidase inhibitory activity; however, unlike the ethanol extracts, the water extract revealed the least capable inhibition due to its high range of concentration. In the ethanol extracts, there

was a dominant inhibitory ability from hot-extracted 96% ethanol extract compared to others at 2.34 $\mu\text{g/mL}$ concentration accounting for 70% inhibition. At 4.69 $\mu\text{g/mL}$, α -glucosidase activity was inhibited over 90% by 96% ethanol extracts, over 70% by 45% ethanol extract by hot extraction, and over 60% by 45% ethanol extract by maceration, while inhibition by aqueous extract was only about 30% (Table 2).

The examination of α -amylase and α -glucosidase inhibitory activity of crude extracts from *E. glaucum* seeds were illustrated to IC_{50} value ($\mu\text{g/mL}$). The IC_{50} value of α -amylase inhibitory activity from these crude extracts was higher than acarbose (0.247 mg/mL). The extracts showed potent α -amylase inhibitory activity with IC_{50} values in the order of $\text{E1} < \text{E3} < \text{E2} < \text{E4} < \text{E5}$ (Table 1). In contrast, the IC_{50} value of α -glucosidase inhibitory activity from these crude extracts was several times lower than acarbose (411.80 $\mu\text{g/mL}$). The extracts showed strong α -glucosidase inhibitory activity with IC_{50} values in the order of $\text{E2} < \text{E1} < \text{E4} < \text{E3} < \text{E5}$ (Table 2). 96% ethanol extract by maceration

(0.496 mg/mL) and 96% ethanol extract by hot extraction (1.511 $\mu\text{g/mL}$) were the most efficient in α -amylase inhibitory activity and α -glucosidase inhibitory activity, respectively. On the whole, according to these results, the priority of using 96% ethanol extract by hot extraction (E2) from *E. glaucum* seeds has satisfied the requirements for *in vivo* tests. The sample size of the seed powder was scaled up to obtain a potential extract (Extraction yield is 2.12%) for further tests.

3.2. Antioxidant activity of *E. glaucum* ethanol seed extract

The 96% ethanol extract had antioxidant activity through DPPH free radical scavenging and ABTS radical cation decolorization capacities (Table 3) with IC_{50} values of 10.98 $\mu\text{g/mL}$ and 12.03 $\mu\text{g/mL}$, respectively. Vitamin C had the ability to scavenge 13.25 - 96.04% DPPH radicals and 14.30 - 81.83% ABTS radicals at a concentration range of 1.38-22.02 $\mu\text{g/mL}$ and 1.67-13.33 $\mu\text{g/mL}$ with an IC_{50} value of 4.30 $\mu\text{g/mL}$ and 7.35 $\mu\text{g/mL}$, respectively.

Table 3. *In vitro* antioxidant activity of *E. glaucum* ethanol seed extract

Activity	% Inhibition					IC_{50} ($\mu\text{g/mL}$)
Conc. ($\mu\text{g/mL}$)	1.95	3.91	7.81	15.63	31.25	
DPPH	8.58 $\pm$ 0.13	21.05 $\pm$ 4.36	39.77 $\pm$ 2.34	59.36 $\pm$ 0.85	81.92 $\pm$ 1.03	10.98
Conc. ($\mu\text{g/mL}$)	3.33	8.33	11.67	16.67	25.00	
ABTS	17.08 $\pm$ 1.73	39.34 $\pm$ 6.59	46.02 $\pm$ 7.89	67.36 $\pm$ 1.16	94.27 $\pm$ 2.17	12.03

3.3. Acute oral toxicity of *E. glaucum* ethanol seed extract

The mice administered *E. glaucum* ethanol seed extract at a dose of 19.84 g/kg showed no abnormal symptoms and no lethal mice were recorded during observation time. Thus, the extract did not exhibit acute oral toxicity in mice (*Swiss albino*) with the maximum dose (D_{max}) of 19.84 g/kg. Accordingly, the doses used for effect evaluation in this study were 50 mg/kg, 100 mg/kg, and 200 mg/kg (equivalent to 10 g, 20 g and 40 g of dried medicinal materials), corresponding to a rate of about 1/400, 1/200, and 1/100 D_{max} , respectively.

3.4. Effect of *E. glaucum* ethanol seed extract in OGTT

The results of the oral glucose tolerance of glucose-loaded normal mice carried out on day 1 (single dose of *E. glaucum* ethanol seed extract) and day 7 (repeated doses of *E.*

glaucum ethanol seed extract) are shown in Table 4. At a single dose, the average plasma glucose concentration of the groups was not significantly different from each other at 0 min and 120 min. The mean plasma glucose level of the 50 mg/kg and 100 mg/kg extract administered mouse group decreased slightly compared to the control group at 30 min and 60 min post oral glucose loading ($p > 0.05$). The percentage decrease in plasma glucose compared to the control group was greatest in the 100 mg/kg extract received mouse group after 30 min of glucose tolerance ($\downarrow 17.97\%$) ($p = 0.43$). Meanwhile, the extract at the dose of 200 mg/kg did not show an anti-hyperglycemic effect in this test. In contrast, glibenclamide, a hypoglycemic agent, showed a significant effect after 60 min of glucose overload with a percentage of glucose lowering of 36.89% compared to the control ($p = 0.02$).

Table 4. Effect of *E. glaucum* ethanol seed extract on plasma glucose level in hyperglycemic mice by glucose loading

Administration	Group	Plasma glucose level (mg/dL)			
		0 min	30 min	60 min	120 min
Single dose	Control	88.74 ± 19.09	187.55 ± 22.37	140.31 ± 29.96	75.00 ± 13.50
	Extract 50	87.15 ± 19.33	176.88 ± 42.25 (↓5.69%)	120.75 ± 20.16 (↓13.94%)	77.17 ± 17.20
	Extract 100	91.11 ± 13.26	153.85 ± 33.14 (↓17.97%)	119.27 ± 13.49 (↓15.00%)	86.17 ± 13.91
	Extract 200	113.54 ± 24.26	196.84 ± 43.60	144.07 ± 49.14	98.62 ± 22.48
	Glibenclamide	97.33 ± 12.85	130.70 ± 19.49 (↓30.31%)	88.55 ± 20.53 (↓36.89%)	73.72 ± 12.97
Repeated doses	Control	95.68 ± 24.08	193.39 ± 21.43	145.15 ± 33.18	98.92 ± 31.85
	Extract 50	105.19 ± 17.96	140.62 ± 21.82 (↓27.29%)	125.25 ± 12.18 (↓13.71%)	109.28 ± 4.37
	Extract 100	107.58 ± 32.04	158.98 ± 21.22 (↓17.79%)	130.84 ± 21.27 (↓9.86%)	106.19 ± 24.09
	Extract 200	92.61 ± 18.11	174.75 ± 24.40 (↓9.64%)	133.83 ± 24.03 (↓7.80%)	102.59 ± 21.29

Mean of the plasma glucose level of mouse groups administered with the *E. glaucum* ethanol seed extract (50, 100, and 200 mg/kg/day, *p. o.*) at a single dose or glibenclamide (5 mg/kg/day, *p. o.*) and repeated doses of the extract for 7 days (Mean ± SD, *n* = 6). (↓ a%) indicates a percentage reduction in plasma glucose level compared to control at the same time. **p* < 0.05 and ***p* < 0.01 compared to control using One-way ANOVA followed by the Turkey test.

After 7 days of treatment with the extract, the degree of reduction in plasma glucose compared to the control was relatively better than that of the single-dose treatment. The mean plasma glucose level in the group of mice administered with the extract at the dose of 50 mg/kg was significantly reduced by 27.29% compared to the control group after 30 min of glucose overload (*p* = 0.003). The extract at the dose of 100 mg/kg reduced plasma glucose by 17.79% and 9.86%

after 30 min and 60 min of glucose overload compared with the control group, respectively (*p* = 0.06 and *p* = 0.73, respectively). On the other hand, the extract at the dose of 200 mg/kg showed a lower reduction in plasma glucose with the rate of 9.64% and 7.80% compared to the control group at these two time points (*p* = 0.48 and *p* = 0.84, respectively). Since glibenclamide has demonstrated efficacy in a single dose, this regimen should not be used.

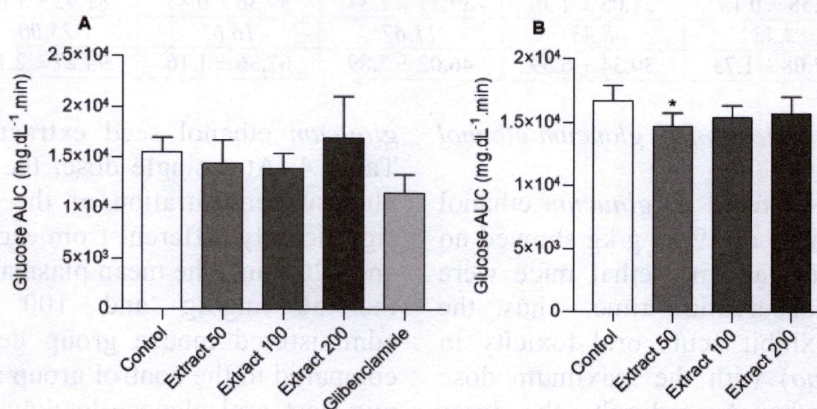


Fig. 1. Area under the curve (AUC) of glucose of mouse groups. Mean of AUC of mouse groups administered with *E. glaucum* ethanol seed extract (50, 100, and 200 mg/kg/day, *p. o.*) or glibenclamide (5 mg/kg/day, *p. o.*) at single dose (A) and repeated doses of the extract (B) for 7 days (Mean ± SD, *n* = 6). **p* < 0.05: compared to control using One-way ANOVA followed by the Turkey test.

The AUC of the mouse group is shown in Fig. 1. The results showed that the extract at the dose of 100 mg/kg (both single dose and repeated doses) was able to reduce the AUC of glucose compared to the control group, but there was no statistically significant difference (*p* = 0.78 and *p* = 0.22, respectively). The extract at the dose of

200 mg/kg showed only a slight decrease in the AUC of glucose after 7 days of oral administration compared to the control group (*p* = 0.41). Interestingly, the extract at the dose of 50 mg/kg significantly reduced glucose AUC compared to the control after 7 days of oral administration (*p* = 0.02). Glibenclamide at a

single dose of 5 mg/kg slightly reduced glucose AUC compared to the control group ($p = 0.06$).

4. Discussion

Many factors that related to diabetes mellitus, one of them is related to α -amylase and α -glucosidase. α -Amylase can be found in saliva, and pancreas glands and is a critical component in the degradation of dietary carbohydrates (normally starch) into monosaccharides for the digestive system. This absorbable form is then further broken down into glucose by α -glucosidase and finally enters the bloodstream. So, the inhibition of α -amylase could prevent the hydrolytic carbohydrate, and α -glucosidase could prevent absorption of these substances into the blood vessel. In this way, these two enzymes could not function normally, so it leads to suppress digestion and delay glucose uptake and then reduces the blood sugar levels, that impact notably on diabetes mellitus type 2 treatment [17]. Acarbose which is known as an α -amylase and α -glucosidase inhibitory drug has been used in certain guidelines for treating diabetes. Acarbose also does not increase blood insulin, does not proceed to insulin resistance, preserves β -cells, reduces triglyceride levels, and reduces complications of diabetes. Nevertheless, acarbose's gastrointestinal side effects and many risks of other synthetic drugs are still a big concern for many people [6].

In this study, *in vitro* inhibition of α -amylase and α -glucosidase activity by 45% and 96% ethanol extracts by maceration, 45% and 96% ethanol extract by hot extraction, and water extract by decoction from *E. glaucum* seeds was conducted to screen for potential extract. The results indicated that all extract types of *E. glaucum* seeds are promising α -amylase and α -glucosidase inhibitors. The 96% ethanol extract by maceration gave the best effect on inhibiting α -amylase and 96% ethanol extract by hot extraction in inhibiting α -glucosidase. In particular, 96% ethanol extract by hot extraction compared to acarbose has shown an impressive potential in inhibitory α -glucosidase activity. Our data therefore provided the scientific basis for the claimed use of *E. glaucum* seeds for diabetes control. Simultaneously, our results were also consistent with previous studies showing α -amylase and α -glucosidase activity related to *Ensete superbum* seeds with ethanol extract [9], *Musa balbisiana* Colla seeds with acetone extract [18]. Notably, for α -glucosidase inhibition, the ethanol extracts of 96% and 45% in our study had

lower IC_{50} values (i.e. stronger activity) than the 70% ethanol extracts from *E. glaucum* seeds (5.71 μ g/mL), pulps (110.33 μ g/mL), and peels (91.23 μ g/mL) [19], and the *E. superbum* seed methanol extract (1.80 μ g/mL) [9] in the previous report. There are various reasons to clarify the difference in each type of solvent, which is related to temperature in extraction methods or polarity of solvent (i.e. different extraction methods lead to different chemical compositions as well as their content, resulting in different biological activities). Phytochemicals are also important components responsible for the inhibitory effects of these two enzymes [20]. Several studies on *E. glaucum* and species related to *E. glaucum* have shown that they include polyphenols, flavonoids, saponins, and tannins [11, 21]. This suggests that furthermore, research on compounds in *E. glaucum* seeds that have the ability to inhibit α -glucosidase and the mechanism of this enzyme inhibition is essential to clarify their effect, for instance, *in silico* molecular docking studies.

Prolonged hyperglycemia leads to oxidative stress that causes cellular damage and participates in the development of diabetic complications [22]. In nature, many parts of different herbs contain abundant natural antioxidants such as tannins, flavonoids, vitamins C and E, which are involved in maintaining β -cells performance and reducing blood glucose levels [23]. In the present study, the free radical scavenging activity of the hot-extracted 96% ethanol extract was also found to show its potential in combating oxidative stress to support the treatment of diabetes; however, further *in vivo* studies are needed.

The OGTT is a good and cheap measure of insulin secretion, sensitivity, and glucose uptake. The test measures an individual's ability to utilize ingested glucose over a given period of time. For normal tolerance to blood glucose, blood glucose concentration at 2 hours following oral glucose loading should return to normal, whereas for impaired tolerance, blood glucose will be higher due to lack or insensitivity of insulin, resulting in reduced glucose uptake and its utilization by the muscle and adipose tissues. As impaired oral glucose tolerance indicates the predisposition of an organism to diabetes, medicinal plants that possess antidiabetic actions will improve glucose tolerance, thereby stopping the progression of impaired glucose tolerance to diabetes [24]. The findings of this study indicated that 96% ethanol extract by hot extraction from *E. glaucum* seeds

at 50 mg/kg exhibited a potential for lowering plasma glucose. The glucose area under the curve (AUC), which is an index of whole glucose excursion after glucose loading, has been widely used for evaluating the efficacy of medications for postprandial hyperglycemia [25]. The results showed that pretreatment of normal mice for 7 days with the *E. glaucum* seed extract at the doses of 50 mg/kg induced a significant reduction in AUC relative to the control group.

In this study, preliminary results about the efficacy of *E. glaucum* seeds for postprandial hyperglycemia were presented, suggesting that *E. glaucum* seeds may be a potential candidate for further research. Besides, the results of the oral glucose tolerance test showed that the effect of the extract tended to decrease as its dose increased; therefore, dose reduction in subsequent investigations is required.

5. Conclusion

The extracts of *Ensete glaucum* seeds inhibited successfully the α -amylase and α -glucosidase activity in the *in vitro* assay and surpassed that of acarbose in α -glucosidase inhibition. The 96% ethanol extract by hot extraction had very strong α -glucosidase inhibitory activity as well as DPPH and ABTS radical scavenging properties. The extract also caused no acute toxicity in mice and was able to reduce plasma glucose in mouse oral glucose tolerance tests. This states a fundamental and reinforcing source for upcoming research navigation.

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STUDY ON THE ANTI-HYPERGLYCEMIC EFFECT OF *HIBISCUS TILIACEUS* L. LEAVES IN OBESE/DIABETIC (+DB/+DB) MICE

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Summary

Study on the Anti-Hyperglycemic Effect of *Hibiscus tiliaceus* L. Leaves in Obese/Diabetic (+DB/+DB) Mice

Diabetes mellitus is considered as the main cause of burning the global health system every year. This study aims to contribute to this growing area of research by investigating the anti-hyperglycemic effect of 70% ethanol extract from *Hibiscus tiliaceus* L. leaves at the oral doses of 0.52 g/kg and 1.04 g/kg (equivalent to 2.5 g and 5 g absolute dry weights of raw materials per kilogram mouse body weight) in the high fatty diet-induced obesity mice with diabetes mellitus by streptozotocin agent (HFD+STZ). After 14 days of oral treatment, the results showed that the 70% ethanol *H. tiliaceus* L. leaves extract at the oral doses of 0.52 g/kg and 1.04 g/kg decreased the blood glucose level significantly in comparison with pathological control (HFD+STZ). This effect of the 70% ethanol *H. tiliaceus* L. leaf extract at the oral dose of 1.04 g/kg was better than the reference drug metformin (500 mg/kg). Moreover, it reduced triglyceride in the plasma significantly in comparison with pathological control (HFD+STZ). According to this study, there was only one 70% ethanol *H. tiliaceus* L. leaves extract at the oral dose of 1.04 g/kg lowered total cholesterol significantly as compared with pathological control (HFD+STZ). All these that *Hibiscus tiliaceus* L. leaves extract might be potentially in the supportive treatment for the non-insulin-dependent diabetes mellitus in obesity patients in the future.

Keywords: *Hibiscus tiliaceus* L. Leaves, Anti-hyperglycemic, Diabetes mellitus, High-fat diet, Streptozotocin.

1. Introduction

The rapid development of modernization, urbanization, and accelerated socio-economic growth favored an improved living standard but a more stressful and sedentary lifestyle and unhealthy dieting habits in most parts of the world. Especially in the last two decades, obesity has become a global pandemic threatening people's lives by affecting almost every organ system and is now a severe public health problem as one of the most common non-communicable diseases (NCDs). With no exception, all countries are now being affected by obesity, and this impact is predicted to be even more prominent during the current decade, resulting in

more lost years of a healthy life, disability, and death. Hence, it is urgent to make effective and decisive actions to hinder the rise in the prevalence of obesity to prevent and treat obesity and other obesity-related comorbidities, among which type 2 diabetes mellitus (T2DM) is a health issue growing at an alarming speed in all regions as another global health emergency of the 21st-century. The booming increase in the prevalence of obesity in all age groups is one of the main culprits of the exponential growth of the population of T2DM [1].

Hibiscus tiliaceus L. is a scientific name of the plant that depends on the *Hibiscus* genus, Malvaceae family. It is also called by the