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Inhibition of Edible Plant Torch Ginger (*Etlingera elatior* (Jack) R. M. Sm.) against α-Glucosidase and α-Amylase

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ABSTRACT: Indonesia's diabetes cases were in the top ten list globally, with 90% of the patients being type-2 diabetes mellitus (T2DM). An approach for screening the local edible plants is made for managing or treating T2DM by inhibiting α-glucosidase and α-amylase enzymes. Results showed that inflorescence of *Etlingera elatior* inhibition toward both enzymes. TLC-autography and IC_{50} value of fractions from *E. elatior* were used to identify the potential fractions and possible compounds for the activity. The non-polar fraction was spotted in the active substances based on TLC-autography. Then, the targeted compounds were separated by column chromatography to obtain stigmasterol as one of the active compounds. The IC_{50} values of total extract, *n*-hexane, EtOAc, *n*-BuOH, and isolated compound against α-glucosidase were 16.0, 7.5, 13.5, 9.7, and 2.0 ppm, respectively. The IC_{50} values for α-amylase inhibition were respectively 88.6, 48.6, 23.2, 29.1, and 27.5 ppm. The positive control (acarbose) against α-glucosidase and amylase exhibited IC_{50} values of 153.2 and 12.3 ppm. The inhibition of *E. elatior* against the two enzymes could be an alternative to delay carbohydrate absorption.

Keywords: torch ginger; type-2 diabetes mellitus; anti-diabetes; TLC-autography.

Introduction

International Diabetes Federation (IDF) mentioned that the population of diabetes people is 1 in 10, and these cases also increased in adults [1]. Indonesia was in the top ten countries listed with diabetes, and 90% of the diabetic people were type-2 diabetes mellitus (T2DM). Every province in Indonesia showed an increasing number of diabetics, with the impact of people from middle to lower economic income [2,3]. Since T2DM can be managed by diet, searching for the potential plants for anti-diabetes is a crucial step [4]. This approach can be helpful for diabetic people to manage their normal glucose levels with action of antidiabetic drugs is inhibition toward α-glucosidase and α-amylase enzymes. This mechanism of action of those enzymes can reduce the absorption of carbohydrates in the intestine by preventing the breakdown of carbohydrate complexes into glucose [5,6]. However, Indonesian people consume more carbohydrates every day, so this mechanism of action can be applicable.

In a previous study on screening local edible plants, fourteen methanolic extracts from edible plants were selected for TLC-autography against *a*-glucosidase inhibition [7]. One of the active plants was the inflorescence of *E. elatior*, which exhibited active substance on the screening with the eluent $CHCl_3$:EtOAc:MeOH (65:20:15). This plant is well known as a vegetable in Asian countries, local people in West Sumatra call it with *kincuang* or *sambuang*. In this research, the selected plant was extracted and fractionated with non-polar, semi-polar, and polar solvents. After that, the fractions were checked for the active fraction by TLC-autobiography and determined their IC_{50} against *a*-glucosidase. In addition, the assay was also submitted to *a*-amylase inhibition.

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Methods

Materials

All the solvents, such as *n*-hexane, EtOAc, MeOH, and *n*-BuOH were distilled. The $CHCl_3$ p.a. and MeOH p.a were used for this research. Anisaldehyde (ANS) was used as a spray reagent of non-active UV compounds.

The assays used enzyme *a*-glucosidase (Sigma, United States) and enzyme *a*-amylase from *Bacillus licheniformis* (Sigma, United States), *p*-naphthyl-*a*-D-glucosidase (sigma, United States), Fast Blue salt (Sigma, United States), phosphate buffer solution: NaH_2PO_4 (Merck, Germany), Na_2HPO_4 (Merck, Germany), DMSO, ethanol p.a (Merck, Germany), starch (Merck Millipore, Germany), $KNaC_4H_4O_6.4H_2O$ (Merck, Germany), 3,5-dinitrosalisicylic acid/DNSA (Himedia), NaOH p.a (Merck, Germany), NaCl (Merck, Germany), DMSO, aquabidest. Acarbose (Sigma, United States) was used as a positive control.

Sample Collection

E. elatior inflorescent purchased in the traditional Guguak, Solok District, West Sumatra market.

TLC-Autography

Thin layer chromatography (TLC) plate silica gel 60 F_{254} (Merck, Germany) was done using $CHCl_3$:EtOAc:MeOH (65:20:15) as eluent followed previous research [7]. TLC-Autography followed previous studies [7] and Simoes-Pires with slight modification [8]. Each extract and fraction were dissolved in MeOH at a 10 mg/mL concentration and spotted 8 μL with capillary micropipette on the TLC plate.

The α-Glucosidase Inhibition Activity

Each extract was dissolved in DMSO with final concentrations 125, 62.5, 31.25, 15.63, 7.81, 3.91, and 1.95 ppm in a mixture of phosphate buffer solution (PBS) and DMSO 1%. Isolated compound and acarbose were prepared at 200, 100, 50, 25, 12.5, 6.25, 3.13, and 1.56 ppm concentrations. Aliquots of 50 μL of the sample (triplicated) and 50 μL enzyme (0.26 U/mL) were placed in a 96-well microplate and incubated for 10 min at room temperature (27°C). Then, 100 µL of *p*-nitrophenyl-*a*-D-glucopyranoside (dissolved in phosphate buffer solution, pH 6.9) was added and incubated for 20 min at room temperature. The absorbance was measured at 405 nm in microplate reader (Allsheng®, China). The control for the sample consists of the sample without enzyme. The blank comprised a phosphate buffer solution with enzyme and substrate (Ac). As referred, absorbance sample minus absorbance control of sample [9].

% inhibition = (1-As/Ac) x 100%

The α-Amylase Inhibition Activity

The *a*-amylase inhibition assay was performed using the 3,5-dinitrosalicylic acid (DNSA) [10] following the method described by Wickramaratne with slight modification [11]. Each sample stock solution was dissolved and prepared at a concentration of 10.000 ppm for extracts and acarbose 1000 ppm for isolated compounds. Each sample was diluted eight times in PBS with DMSO 10%. Aliquots of 100 μL of sample (triplicate) were incubated with enzyme (10 U/mL) at room temperature for 10 min. Then, 100 μL of the substrate (starch 1% dissolved in PBS) was added and incubated at room temperature for 3 min. After the incubation, stop the reaction by adding 100 μL DNSA and heating at 105°C for 20 min, then add 500 μL aquabidest. The absorbance was measured at 540 nm [11]. The control for the sample consists of the sample without enzyme. The blank comprises a phosphate buffer solution with enzyme and substrate (Ac). As referred to, absorbance sample minus absorbance control of the sample.

% inhibition = 1-As/Ac) x 100%

Isolation of Targeted Compounds from Non-Polar Fraction

Fresh *E. elatior* inflorescence (10 Kg) was chopped into small pieces and macerated with MeOH for 3 x 72 h. Each maceration was filtered and combined, evaporating inrotary evaporator (Buchi®, Switzerland) until it gained 92.1 g. After evaporation, the extract was fractionated using *n*-hexane, EtOAc, and *n*-BuOH, respectively. All fraction was evaporated in a vacuum using a Rotary evaporator to obtain *n*-hexane, EtOAc, and *n*-BuOH extracts.

The dry load of *n*-hexane fraction was prepared by dissolving the *n*-hexane extract (12 g) with EtOAc and then adding 24 g silica gel. The silica column chromatography was packed with a slurry of 480 g silica gel 60 F_{254} (Merck KGa®, Germany) in *n*-hexane. The column was eluted by step gradient polarity of *n*-hexane 100%, *n*-hexane-EtOAc (4:1, 3:2, 1:1, 2:3, 1:4), EtOAc 100%, EtOAc: MeOH (3:2, 1:1), and MeOH 100%. The results were collected in vial and monitored using a TLC plate with eluent $CHCl_3$:EtOAc:MeOH (65:20:15). The targeted fractions were combined, which were vial 50-165 for sub-fraction C and vial 166-260 for sub-fraction D. Sub-fraction C was separated using silica column chromatography with isocratic eluent *n*-hexane:EtOAc (9:1) then the isolated

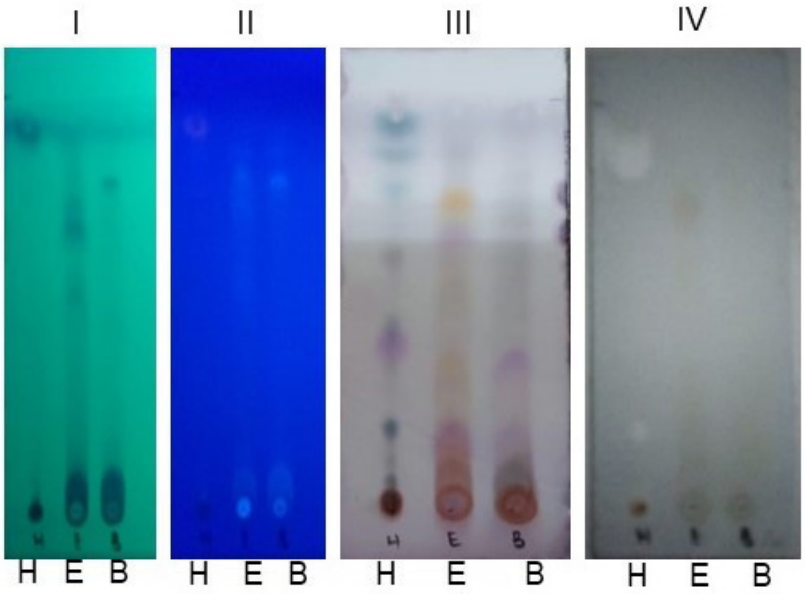

Figure 1. Results of TLC-autography of *n*-hexane (H), EtOAC (E), and *n*-butanol (B) fractions, **I**. TLC visualization under UV 254 nm, **II**. TLC visualisation under UV 366 nm, **III**. TLC plate after spraying with ANS reagent, **IV**. TLC plate after spray with α-glucosidase enzyme, white spots indicated the active substances inhibition toward α-glucosidase

compound was recrystallized with *n*-hexane and EtOAc to obtain compound **1**. Sub-fraction D was prepared similarly to sub-fraction C with EtOAc 100% as eluent and recrystallized in EtOAc and MeOH to gain compound **2**.

Identification of Isolated Compounds

Both isolated compounds were identified using LC ACQUITY UPLC and MS: Xevo G2-S QTof (Waters, United States) with C18 column (1.8 µm, 2.1x100 mm) [temperature at 25°C, flow rate 0.2 mL/min for 23 min, eluent water + ammonium format 5mM (A) and acetonitrile + formic acid 0.05% (B)], spectrophotometer UV-Vis at 200-800 nm (Shimadzu®, Japan), spectrophotometer FT-IR (Perkin Elmer, United States), and melting point apparatus Melting Point Stuart SMP30 (Cole-Palmer, United States).

Data Analysis

The IC_{50} calculation of *a*-glucosidase and *a*-amylase used linear regression and Excel probit analysis.

Result and Discussion

Screening local edible plants for anti-diabetes would increase the scientific evidence on traditional medicine for α-glucosidase inhibition later. The selected extract was from inflorescent of *E. elatior*, then fractionated with different polarity solvent. The extraction process obtained total extract, *n*-hexane, EtOAc, and *n*-butanol fractions of 92.1, 15.0, 10.0, and 17.0 g, respectively. TLC-autography screened all fractions based on the previous method [7]. The TLC-autography was depicted in Figure 1, which showed two white spots after spraying with substrate, α-glucosidase, and fast blue salt [8]. The white spot indicated the inhibition of substance against *a*-glucosidase. This method was simple, fast, effective, and compatible for *a*-glucosidase inhibition. The result guided the active compounds were in non-polar fraction, *n*-hexane fraction.

Identification of the responsible compounds for the activity was continued by doing isolation steps [12,13]. Silica column chromatography was used to separate the substances from the *n*-hexane fraction. The eluent from previous research [7] isolated the target compounds. The pure isolated compound was obtained in amounts of 72.8 mg for compound **1** and 10 mg for compound **2**. However, compound **2** cannot be completely identified and tested because of its limited quantity. Compound **1** was identified as stigmasterol based on LC-MS data with ion *m/z* $[M+H]^+$ 413.2647, same as stigmasterol [14]. The Infrared spectrum and melting point of compound **1** was the same as the reference [15]. The infrared spectrum showed the hydroxyl group at 3428 cm^{-1}, C-H group at 2937 cm^{-1}. The measured melting point was 168.3-170.9°C while the literature was 169-171°C. The UV spectrum exhibited λ max at 212.5 nm.

Maintaining the glucose level is essential for diabetes

Table 1. The IC_{50} value of each sample

No.	Sample	IC_{50} for α-glucosidase inhibitor (ppm)	IC_{50} for α-amylase inhibitor
1.	Total extract	16.0	88.6
2.	*n*-hexane extract	7.5	48.6
3.	EtOAc extract	13.5	23.2
4.	*n*-BuOH extract	9.7	29.1
5.	Compound 1	2.0	27.5
6.	Acarbose	153.5	12.3

patients to reduce complications and mortality [16]. Socioeconomic issues can cause complications. In this case, inhibition towards enzymes responsible for glucose metabolism and absorption is essential. The inhibition values against *a*-glucosidase and *a*-amylase were calculated for all extracts and compound **1**. Based on the assay from those two enzymes, *a*-glucosidase inhibition from all extracts and compound **1** of *E. elatior* had more potential than acarbose in Table 1. However, *a*-amylase inhibition of acarbose was higher than all extracts and compound **1** from *E. elatior*. Stigmasterol was also reported from seaweed and fruit of *Morinda citrifolia* as anti-diabetic through inhibition against *a*-glucosidase and *a*-amylase [17,18].

Conclusion

The edible part of E. *elatior* could inhibit α-glucosidase and α-amylase activities. The non-polar fraction identified stigmasterol as one of the responsible compounds for the activity through initial TLC-autography method.

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