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Preliminary study: α-glucosidase Inhibition Ethanolic Extract from Pogeh-Pogeh (*Alpinia denticulata* (Ridl.) Holttum)

Nanda Putra[1]*, Jihan Hanifah Al-Amin[1], Fitratul Wahyuni[2]

[1]Departement of Pharmaceutical Biology, Sekolah Tinggi Ilmu Farmasi (STIFARM) Padang
[2]Departement of Pharmacology and Clinical Pharmacy, Sekolah Tinggi Ilmu Farmasi (STIFARM) Padang

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*Corresponding Author :
Nanda Putra
Email: nanda.putra0609@gmail.com

ABSTRACT

α-glucosidase inhibitor is a potential antidiabetic drug in controlling the blood glucose in diabetic patients. This study aims to screen the inhibitory activity of various parts of *Alpinia denticulata* against the α-glucosidase enzyme through *in-vitro* testing. As a preliminary study, the 70% ethanolic extract of the *Alpinia denticulata* including rhizome, stem, leaf, and fruit was evaluated for inhibition of α-glucosidase, together with total phenolic content and the toxicity based on the Brine Shrimp Lethality Test (BSLT). Each part of the plant extract showed a higher IC_{50} value than acarbose as a positive control (IC_{50} of fruit extract = 14.39 µg/ml, IC_{50} of leaf extract 6.13 µg/ml, IC_{50} of stem extract 20.57 µg/ml, rhizome extract 126.67 µg/ml and acarbose 172.02 µg/ml). Interestingly, each of the extract also showed different quantities of total phenolic content in the same order as their IC_{50} in inhibiting α-glucosidase activity. Additionally, the BSLT showed that only the leaf and stem are in the non-toxic group. Based on the assay, it suggested that this plant has the potential to be investigated as an antidiabetic drug.

Keywords : Ginger, A-Glucosidase, Alpinia, Diabetes, Pogeh-Pogeh, Diabetes

INTRODUCTION

One of the metabolic diseases included in the 10 diseases that cause the highest deaths in the world is diabetes (Magliano & Boyko, 2021). This disease is characterized by high levels of glucose in the blood, commonly more than 200 mg/dl if checked two hours after eating and more than 126 mg/dl if checked while fasting (Magliano & Boyko, 2021). This condition is caused by disruption of the performance of the insulin hormone, secretion of this hormone, or both at the same time causing glucose not to be able to enter the cells and tissues of the body optimally and still circulate in the blood (Magliano & Boyko, 2021). If this condition continues for a long period, it can cause damage to various organs such as the eyes (retinopathy), kidneys (nephropathy), nerves (neuropathy), and cardiovascular (Zheng *et al.*, 2018).

Thepopulation of people suffering from diabetes continues to increase from year to year, including in Indonesia (Magliano & Boyko, 2021). Various efforts to control diabetes continue to be carried out and sounded. Multiple efforts to control diabetes continue to be made and expressed, such as maintaining a healthy diet, controlling body weight, regular exercise, and the use of oral anti-diabetic drug therapy (Ministry of Health, 2018).

The use of oral antidiabetic drugs is an unavoidable choice for diabetes patients to control blood sugar levels. One type of oral antidiabetic drug that is available works by inhibiting the action of the enzyme that is responsible for the process hydrolysis of disaccharides into monosaccharides (glucose) in the digestive tract, this enzyme is α-glucosidase (Magliano & Boyko, 2021). Acarbose, miglitol, and voglibose are three drugs from the α-glucosidase inhibitor group that are available on the market currently (Ghani, 2020). Routine use of oral anti-diabetic drugs also turns out to be ineffective in reducing diabetes cases, and this turns out to be accompanied by high costs and common side effects.

Exploration of medicinal plants, especially as α-glucosidase inhibitors from nature, in the search for alternative diabetes therapies continues to be pursued. It is believed that natural resources, particularly plants, may serve as effective α-glucosidase inhibitors with similar therapeutic effects but fewer side effects compared to the oral α-

glucosidase inhibitors currently available (Dirir *et al*., 2022). *Alpinia denticulata* is one of the Zingiberaceae plant family that needs to be explored as it was reported as a new record species in Sumatra in 2019 (Maulidah et al., 2019). It is locally called Pogeh-pogeh and even though it is known, nothing has been stated regarding its traditional use by the local communities where this plant is found. Until now, there has been no reported research regarding their antidiabetic activity, especially in inhibition of the α-glucosidase enzyme from the *A. denticulata*. Therefore, this research aims to evaluate the α-glucosidase inhibitory activity of extracts from each plant part, along with toxicity using the Brine Shrimp Lethality Test (BSLT) method followed by determining the total phenolic content (TPC) of each extract as a preliminary study.

RESEARCH METHOD

Materials

Plants were collected in the Harau Valley, Kecamatan Harau, Kabupaten Limapuluh Kota, West Sumatra. Samples were identified and authenticated by Mrs. Dr. Nurainas at Andalas University, ANDA Herbarium.

Tools

Bioactivity data was obtained using a Microplate reader (ALLSHENG FlexA-200), 96 well plate (Biologix), Micropipette (Eppendorf®, Huawei, Dragon), pH meter (Mettler Toledo®), rotary evaporator (Heidolph®), analytical balance (Mettler Toledo®), Erlenmeyer volume 250 mL, 500 mL dan 2 L (Pyrex®), separation funnel, UV 254 nm 365 nm (Camag®), TLC Plate (Merck®), α-glucosidase (Sigma), Acarbose (Sigma), 4-nitrophenyl β-D-glucopyranoside (Sigma)

Extraction

Plant samples were chopped and divided into four parts consisting of rhizome, stem, leaves, and fruit. Each of them was then ground to obtain 570 g rhizome, 1065 g stems, 595 g leaves, and 100 g fruit. Each of them was extracted by maceration method using 70% ethanol (solvent-to-sample ratio 1:10) for 1x24 hours at room temperature.

The resulting macerate was then collected and evaporated under vacuum using a rotary evaporator until a thick extract was obtained for testing from each part, including rhizome extract 8.87 g, stem extract 5.72 g, leaf extract 5.21 g, and fruit extract 3.35 g. The finished extract is then stored at refrigerator temperature before being used for testing. Each extract was then examined for its compound content using a thin layer chromatography (TLC) plate with a prepared mobile phase. In-vitro bioactivity, BSLT, and TPC testing were carried out using each extract.

α-glucosidase Inhibition Bioactivity Assay

This test was carried out based on the method of (Kim et al., 2005) with several modifications to the sample concentration. Rhizome, stem, leaf, and fruit extracts were tested using the same plate with acarbose as a positive control. All samples (150 µg/mL) in separate wells were dissolved in a mixture of 5% DMSO and phosphate buffer pH 6.8, and then 5 mM PNPG substrate was added to the solution. The mixtures were incubated for 5 minutes at 37 °C. The enzyme α-glucosidase was then added to each mixture, and incubation was continued for 15 minutes. The reaction was stopped by adding Na_2SO_3 to each sample, and the absorbance was measured at 405 nm. The percentage of inhibition was calculated using the following equation. % of inhibition :

$$\frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control Absorbance}}$$

The test results were expressed as average % inhibition ± SD.

Total Phenolic Content (TPC)

Total phenolic content was determined using the Folin-Ciocalteu method. 1 ml of each extract was added with 5 ml of 10% Folin–Ciocalteu reagent, then added by 4 ml of NaOH after 8 minutes. The solution was then incubated at room temperature for 1 hour and the absorbance was measured by a Spectrophotometer (Shimazu, UV-1800) at 765 nm and the blank was running without extract. The data is presented as mg/g of gallic acid equivalents in milligrams per gram (mg GAE/g) of dry extract.

Brine Shrimp Lethality Test (BSLT) Method

Approximately 20 mg of Artemia salina leach eggs were placed in a breeding container containing seawater equipped with an aerator and lamp. Then the Artemia salina leach eggs are spread evenly and given light. After 24 hours the shrimp egg larvae will hatch and become active in moving towards the bright aquarium area. The Artemia salina leach larvae used were 48 hours old. 10 shrimp larvae were added to each test extract with concentrations of 1000, 100, 10 µg/mL, and the blank was used as a control. The test was carried out in 3 repetitions and observations were compared with controls for 24 hours.

The percentage of death of Artemia salina shrimp is calculated using the following equation. % of mortality :

$$\frac{\text{Number of dead larvae}}{\text{Number of live larvae}} \times 100\%$$

The LC_{50} value is obtained using the linear regression equation formula y = ax + b. The y value represents the probit value at 50% larval mortality according to the Miller Tainterprobit method, while the x data shows the logarithm value of the test environment concentration. The LC_{50} value is obtained from the concentration (µg/ml) of the solution that causes the death of 50% of the larvae.

RESULTS AND DISCUSSION

A.denticuata is a flowering plant that belongs to the family of Zingiberaceae and this genus consists of 241 accepted species names (The Plant List, 2024). The freshly collected plants were processed following the procedures outlined in the materials and methods section. Maceration is a conventional technique that is used commonly in the extraction of secondary metabolites from plants. This method was chosen because of some advantages such equipment and working technique used are very simple, operational costs are relatively low, and it can be used to extract thermo-labile compounds because it is done without heating. The extraction results are highlighted in the materials and methods section. Thin Layer Chromatography (TLC) Analysis was used to see qualitatively whether there were differences in the chemical content of each extract (Figure 1). TLC was chosen because it is simple, and easy to use and the analysis results were obtained quickly. Based on the TLC results (Figure 1), there are differences in chemical content between extracts from each plant part and this can be seen from the spots produced by each plant part extract. Then, a quantitative analysis was also carried out on the total phenolic compound of each extract as a step to characterize the extract that will be used in further assay including inhibition of α-glucosidase activity together with toxicity using the BSLT test.

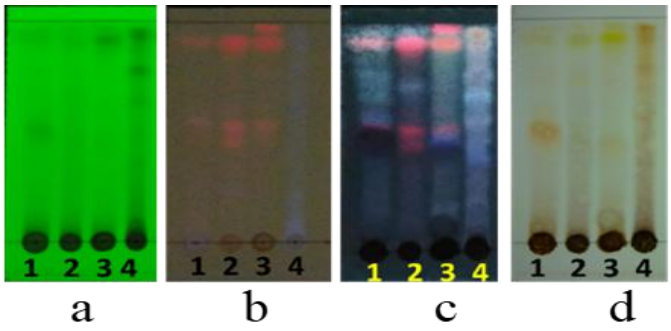

Figure 1. TLC PROFILE of each extract (1:rhizome, 2: stem, 3: leaf, 4: fruit) a. 254 nm, b. 365 nm, c. 365 nm after spray with H_2SO_4 d. under visible light after spray with H_2SO_4

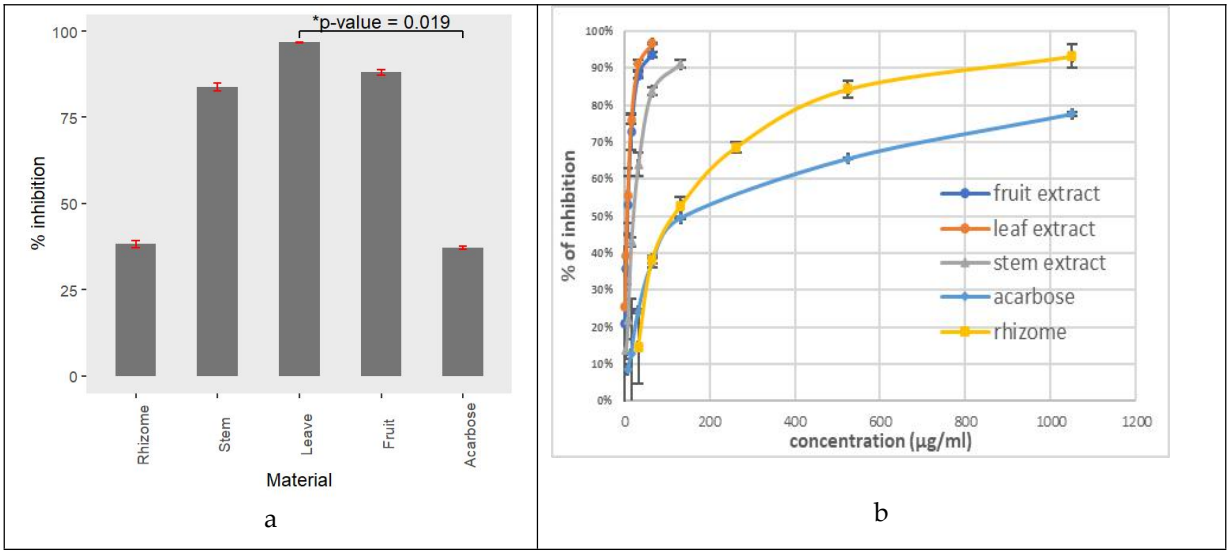

Figure 2. a. Inhibition of α-glucosidase activity at 65.625 µg/ml; b. Inhibition of α-glucosidase activity at various concentration

α-glucosidase inhibition of each extract including fruit, leaf, rhizome, and stem extracts was evaluated compared to acarbose as a positive control as an initial screening. As a result, each extract has a variation in inhibiting the α-glucosidase activity. At the same concentration, 62.625 µg/ml, the highest inhibition was given by leave extract, followed by fruit, stem, and rhizome extract with each % inhibition are 96.71±0.20, 88.04±0.88, 83.73±1.13, 38.28±1.07 respectively, while acarbose at the same concentration gave 37.25±0.48 % of inhibition (Figure 2.a).

These data then analyse statistically using Rstudio software (R Core Team, 2023), Kruskal-Wallis test was performed using α at 0.05 significance level to determine if there is a statistically significant difference between the % of inhibition across these groups (Rhizome, Stem, Leave, fruit, and acarbose). Since the overall p-value (0.01111) is less than 0.05, this means there is a statistically significant difference between the % of inhibition among the 5 groups of samples. Thus, Dunn's test was performed to determine exactly which material is different. At α = 0.05, acarbose and leaves are the only two materials that are statistically significantly different from each other (p-value = 0.019) (Figure 2. a).

The IC_{50} of each extract was then determined and calculated (Figure 2. b**)**, with the result: (IC_{50} fruit extract = 14.39 µg/ml, IC_{50} of leaf extract 6.13 µg/ml, IC_{50} of stem extract 20.57 µg/ml, rhizome extract 126.67 µg/ml and acarbose 172.02 µg/ml). All of the extract has IC_{50} significantly lower than acarbose as positive control and the most commonly prescribed α-glucosidase inhibitor available on the market. Compared to the other natural resources, some plant extracts showed promising potential as an α-glucosidase inhibitors (Yin et al., 2014). For example another plant, research conducted by (Daou et al., 2022) revealed that 50% ethanolic and water extract of *Tamarix nilotica* have IC_{50} values of 12.5 µg/mL and 24.8 µg/mL respectively, below the acarbose as a positive control with IC_{50} = 151.1 µg/mL.

Additionally, each extract also contains different total phenolic compounds (Table1), with the order as the same as IC_{50} from inhibition of α-Glucosidase activity. This result suggests that this group of compounds has a role in the inhibition of α-Glucosidase. The limitation of this study was there is no supporting data to provide the chemical profiling of each extract by LC-MS method, so the information of what compound contains phenolic group cannot be mentioned.

Another study revealed that phenolic compound has an association with inhibition of α-Glucosidase activity by docking simulation study (Swargiary et al., 2023), then (Tanaka et al., 2005) reported that phenolic compound such as galloyl, caffeoyl and *(S)*-hexahydroxydiphenoyl (HHDP) esters of dihydrochalcone glucosides from *Balanophora tobiracola,* has potential activity as α-glucosidase inhibitor with IC_{50} 0.4-1.8 µM. Another plant namely Broussonetia papyrifera, was also reported to contain some phenolic compounds, and chalcone derivatives as potential inhibitors of α-glucosidase with IC_{50} 11.1-19.1 µM (Ryu et al., 2010). To see the toxicity of each extract, a simple and reliable toxicity assay, BSLT has been done to see the toxicity of each extract. Of the four the extract that has been tested, according to (Meyer et al., 1982) only two are in non-toxic category, which are leaves and stem extracts because they have LC_{50} values above 1000 µg/ml (Table 1). Further, another cytotoxic study by (Andania et al., 2019) revealed that extracts of *A. denticulata* rhizome did not show significant activities against the MCF-7 cell line.

Table 1. IC_{50} of inhibition α-Glucosidase, Total Phenolic Content and Brine Shrimp Bioassay Results of Each Extract

No.	Sample	Inhibition of α-Glucosidase activity, IC_{50} (µg/ml)	Total phenolic content (mg GAE/g dry extract)*	BSLT ASSAY	
				LC_{50} in µg/ml	Toxicity category
1	Leaf extract	6.13	37.16% ± 0.01	>1000	non toxic
2	Fruit extract	14.39	31.41% ± 0.02	406.16	Toxic
3	Stem extract	20.57	19.54% ± 0.01	>1000	non toxic
4	Rhizome extract	126.67	8.19% ± 0.01	590.53	toxic

Annotation : *data statistically significant as P < 0.05

CONCLUSION

In conclusion, our study demonstrated that all of the parts *A. denticulata* extracts including the rhizome, stem, leaf, and fruit have activity as an inhibitor of α-glucosidase. However, further research including *in-vivo* studies and isolation of

the active constituents will be interesting to explore to confirm the efficacy of this plant extract.

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