# Antidiabetic Activity of Jering Skin Extract *(Pithecellobium jiringa)* with Dichloromethane (DCM) Solvent Fraction

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**Abstract.** Jering skin is a solid waste that can cause problems to the environment if it is not treated seriously. Based on previous research, Jering skin contains highly flavonoids and polyphenols source which potentially used as an antiseptic and anticancer agent. The study aims to evaluate Jering skin antidiabetic activities using varieties DCM solvent fraction. The microplate reader analysis at 410 nm wavelength was used to measure an absorbance level of the Inhibitor Concentration 50 (IC50) sample extracts which inhibited by the α-glucosidase enzyme when hydrolyzing the p-nitrophenyl substrate α-D-glucopyranoside (p-NPG) to form a glucose molecules. As a result, the Jering skin extract is shown to be an alternative antidiabetic agent in the future, with the IC50 level at 417.381 µg/mL.

## INTRODUCTION

The use of dry skin *(Pithecellobium jiringa)* as organic waste is still lacking, so research into the potential of dry skin as a source of natural ingredients for medicinal products in life is required. In previous studies, researchers have tried to take advantage of ingredients in dry skin, such as ethanol extract from jering skin, which can be used as antibacterial agents against Streptococcus mutants, *Staphylococcus aureus* and *Escherichia coli* [4].

Another study performed by Hutauruk in 2010 was the isolation of flavonoid compounds found in the dry skin itself using a maceration extraction process using methanol as a solvent [2]. Compounds that have potential as antioxidants are usually flavonoids, phenols and alkaloids. Flavonoid and polyphenol compounds are antiseptic, anti-inflammatory and anti-cancer properties. Besides the antioxidant activity in previous studies using the DPPH (1.1-diphenyl-2-picryl hydrazile) method in methanol extract of syringe skin with 72-hour maceration has an IC50 value of 22.5788 ppm, which indicates that the extract has very good activity, and in the Brine Shrimp Lethality Test (BSLT) method, the extract has potential as an anticancer drug with $LC_{50}$ value of 126 ppm [8].

Meanwhile, there has been no definite data on the number of people suffering from diabetes in Indonesia so far. However, it continue to rise. Meanwhile, according to [3] Diabetes mellitus sufferers are at risk of experiencing complications that may affect their quality of life. These problems can be reduced by self-care management (self-management) [3].

WHO reports that the number of diabetics in Indonesia in 2000 was 8.4 million. That figure had increased to 13.8 million in 2003. It is estimated that by 2030 more than 21 million people will suffer. And those suffering from diabetes are also on the rise in Indonesia. This occurs not just in towns, but also in villages. Insulin can be used to treat diabetes mellitus and a mixture of three oral anti-hyperglycemia medications can be used in patients that are accompanied by clinical conditions where insulin is not possible [5].

Oral hypoglycemic medications and herbal treatments. Mechanisms of action of oral hypoglycemic drugs include stimulation of insulin release, an insulin sensitizer, and inhibition of 5-007-glucosidase function. Many traditional medications used to reduce blood sugar levels have some side effects, such as weight gain. However, 5-

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007-glucosidase inhibitors (acarbose, voglibose, and miglitol) do not cause adverse effects, such as hypoglycemia and weight gain, but cause gastrointestinal pain, such as flatulence and diarrhea [1].

# EXPERIMENT

## Materials

The instruments: pH meter, incubator, analytical scale, 10-100 µl (Eppendorf) micropipette, Eppendorf tube, 96 well (epoch) microplate reader, and glass instruments commonly used in laboratories. aqueous, cotton. The materials: α-glucosidase enzyme (Sigma-Aldrich, USA), p-nitrophenyl-α-D-glucopyranose substrate, dimethyl sulfoxide (DMSO), pH 7 phosphate buffer solution, $Na_2CO_3$ solution, n-Hexane, ethyl acetate, methanol.

## Methods

*Preparation of α-Glucosidase Enzyme Inhibition Test Reagent Solution*

Phosphate buffer solution pH 7 is prepared by mixing 1.361 g potassium dihydrogen phosphate into 100 mL solvent. Furthermore, a solution with a pH of 7 is obtained by adding 35 g of disodium hydrogen phosphate to 1000 mL of distilled water. Furthermore, 0.1 M sodium carbonate solution was obtained by dissolving 0.53 g of sodium carbonate in 50 mL of distilled water. To make a 20 mM p-nitrophenyl-α-D-glucopyranoside (p-NPG) substrate solution: A p-NPG substrate solution with a concentration of 20 mM was prepared by weighing 0.1507 g p-NPG and dissolved in 25 mL of phosphate buffer pH 7.

*In Vitro Test for α-Glucosidase Enzyme Inhibition*

In vitro, the inhibition test was conducted with the p-NPG substrate and 5-007-glucosidase enzyme in which the p-NPG substrate is hydrolyzed by the 5-007-glucosidase enzyme to 5-007-D glucose and p-nitrophenol (yellow), the test samples respectively-Each add-on to the substrate and enzyme mixture is required to inhibit the activity of the enzyme to decrease the formation of glucose and intensity. The inhibitory function of this enzyme was modified based om the yellow color of the reaction using a spectrophotometer at a wavelength of 405 nm[6].

*Blank (B0) and (B1) Absorbance Measurement ($B_1$)*

A total of 10 µL of DMSO was applied with 50 µL of phosphate buffer pH 7 and 25 µL of p-NPG 20 mM as (B0) and 10 µL of DMSO plus 50 µL of phosphate buffer pH 7. 25 µL of p-NPG and 25 µL of p-NPG-Glucosidase 0, 2 U / mL as (B1) each combined in a 96-well microplate and incubated at 37 0C for 30 minutes. The reaction was halted by inserting 100 µL 0.1 M Na2CO3 and the absorbance was then measured with a microplate reader at a wavelength of 405 nm[9].

*Measurement of Sample Absorbance (S0) and (S1)*

A total of 10 µL of the sample with two-fold doses of 1000-31.25 µg / mL in DMSO added with 50 µL of phosphate buffer pH 7 and 25 µL p-NPG 20 mM as S0, and 10 µL of the sample with two-fold doses of 1000-31.25 in DMSO added with 50 µL of phosphate buffer pH 7.25 µL p-NPG and 25 µL Δ-Glucosidase 0.2 U / mL as S1, each mixed in a 96-well microplate and incubated for 30 µL p-NPG using Equation 1.

$$\% \text{ inhibition} = \frac{(B_1 - B_0) - (S_1 - S_0)}{(B_1 - B_0)} \text{ x } 100 \quad (1)$$

Note
(B1-B0) = Absorbance of no sample
(S1-S0) = Absorbance of sample

The $IC_{50}$ value is calculated using the logarithmic equation (Y = a lnX + b) of the calibration curve by plotting the sample concentration vs the% inhibition value. All measurements were carried out three times [9].

## RESULTS AND DISCUSSION

The use of dry skin (Pithecellobium jiringa) as organic waste is still lacking, so research into the potential of dry skin as a source of natural ingredients for medicinal products in life is required. In previous studies, researchers have tried to take advantage of ingredients in dry skin, such as ethanol extract from jering skin, which can be used as antibacterial agents against Streptococcus mutants, Staphylococcus aureus and Escherichia coli [4]. Measurement and data analysis results obtained from the data are seen in Table 1.

**TABLE 1**. $IC_{50}$ Value

| Sample | Dose (µg/mL) | Ln Concentration | % Inhibition | $IC_{50}$ (µg/mL) |
|---|---|---|---|---|
| DCM Fraction | 1000 | 6.9077 | 65.471869 | 417.381 |
| | 500 | 6.2146 | 51.361162 | |
| | 250 | 5.5214 | 41.969147 | |
| | 125 | 4.8283 | 29.401089 | |
| | 62.5 | 4.1351 | 20.825771 | |
| | 31.25 | 3.4420 | 12.794918 | |
| Acarbose | 10 | 2.3025 | 83.4392 | 0.8135 |
| | 5 | 1.6094 | 74.0018 | |
| | 2.5 | 0.9162 | 68.3303 | |
| | 1.25 | 0.2231 | 56.2159 | |
| | 0.625 | -0.47 | 46.8693 | |
| | 0.3125 | -1.1631 | 35.2994 | |

An antidiabetic inhibition examination of the enzyme 5-007 glucosidase was performed. Measurement of the uptake of the substance, i.e. p-nitrophenol at a wavelength of 410 nm using a UV-Vis spectrophotometer. Tests were conducted on blank solution (B1), blank control (B0), sample (S1), control sample (S0), root base (A1), and rootbose (A0) control comparators.

Analysis of blank solution (B1) and blank regulation (B0) is done for the enzyme activity without the addition of an extract. The blank solution (B1) and blank control (B0) experiments are typically carried out every day of testing, as the storing of enzyme solutions will lead to a decrease in enzyme activity. Blank (B1) is a reaction mechanism in the absence of an extract that produces a dense yellow hue. Without the inclusion of the extract/sample, the substrate (p-NPG) will be completely hydrolyzed by the 5-007 glucosidase enzyme, resulting in a deeper yellow hue. The yellow color created is an indication of the ability of the sample to act as a reaction inhibitor.

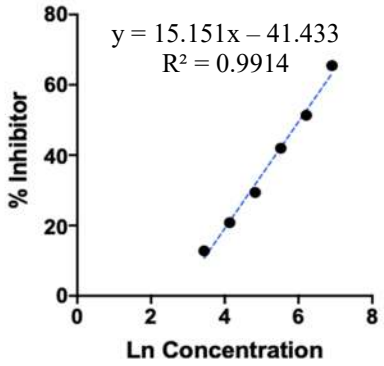


**FIGURE 1.** Percent Inhibition of the 5-007-glucosidase enzyme with a concentration of the DCM fraction

Calculations of $IC_{50}$ (X) for the DCM fraction based on the percent Inhibition of the 5-007-glucosidase enzyme with a concentration of the DCM fraction as shown above is 417.381 ppm.
As for the root compound, the bose is as follows:

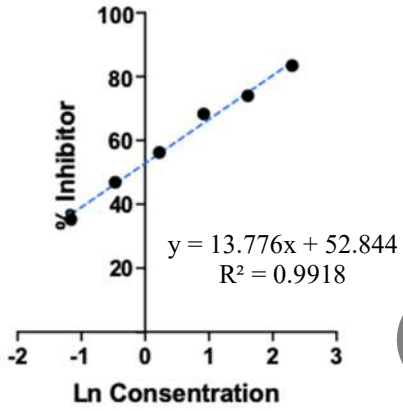


**FIGURE 2**. Percent inhibition of the acarbose concentration-glucosidase enzyme

Based on the percent inhibition of the acarbose concentration-glucosidase enzyme, calculation of $IC_{50}$ (Y) as shown above is 0.813 ppm.The substance produced is directly proportional to the yellow color formed and inversely proportional to the inhibitor's ability to inhibit the product. Testing of the sample solutions is to determine the ability of the sample to inhibit the enzyme 5-007 glucosidase, while testing of the sample control is conducted as a correction factor for the sample solution [7].

The results showed that the acarbose compound had an inhibitory effect on the enzyme Δ glucosidase. Percent inhibition was expressed, the maximum value at a concentration of 10 µg/mL was 83.4392 percent and the IC50 value was 0.8135 µg/mL. Meanwhile, the test results of the syringe bark extract samples isolated by maceration for 72 hours against the 5-007-glucoside enzyme showed the maximum percent inhibition of the 5-007-glucosidase enzyme at a concentration of 1000 µg/mL at just 65.471869 with an IC value of 417.381 µg/mL. From the contrast of the IC50 value between acarbose and the positive control of the extracted sample, this gives a very significant difference, which provides knowledge that the distilled compound can have excellent inhibition relative to the extracts, while the IC50 value of the methanol extract of jering bark is still graded as active since it is still below 500 µg / mL for the compounds.

## CONCLUSION

Based on the results, it was concluded that the extract of the DCM fraction of dry skin could be antidiabetic with an IC50 value of 417.381 µg/mL which was far lower than the pure acarbose compound with an IC50 value of 0.8135 µg/mL.

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