**In vitro** study of *Cratoxylum glaucum* Stem ethyl acetate extract as antidiabetic

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**Abstract.** Diabetes mellitus (DM) is a disease characterized by hyperglycemia due to decreased insulin secretion, or decreased insulin sensitivity, or both. If insulin is not available or the amount is low, then glucose cannot enter the cells and will continue to be in the bloodstream. DM can cause chronic complications such as eye complications, skin infections and stroke. DM treatment takes a long time by using synthetic drugs that will cause side effects, therefore the search for herbal-based drugs is the community's choice. Idat plant (*Cratoxylum glaucum*) is a local plant from Bangka Belitung. Several studies on the genus *Cratoxylum* from China and Thailand showed a very high inhibitory ability against the inhibition of α-glucosidase enzymes, protein tyrosine phosphate, and carbohydrate hydrolysis enzymes because they contain phenolic compounds anthraquinones and xanthones. Therefore, the importance of more intensive research on *C. glaucum* species regarding the content of secondary metabolites in *C. glaucum* stems and their antidiabetic power. The extraction method in this research is maceration with ethyl acetate solvent. Qualitative examination of phenolic and flavonoid content using reagents, as well as antidiabetic testing was carried out in vitro with *dengan* α-glucosidase enzyme inhibition method. Based on qualitative testing of phenolic and flavonoid compounds, the content of these compounds was obtained. Antidiabetic test showed that the *α*-glucosidase inhibitor of ethyl acetate extract had very strong activity with an IC₅₀ of 4.21 g/mL. Therefore, the stem of the idat plant can be used as a therapy for the treatment of diabetes mellitus.

1. **Introduction**

Diabetes mellitus (DM) is a non-communicable disease, which is characterized by hyperglycemia due to decreased insulin secretion, or decreased insulin sensitivity, or both. The hormone insulin is produced by the pancreas to regulate the balance of blood sugar levels [1]. If insulin is not available or the amount is low, glucose cannot enter the cells and will continue to be in the bloodstream [2]. Symptoms experienced by diabetics include polyuria, polyphagia, and polydipsia, and are accompanied by complaints of blurred vision, tingling in the hands or feet, itching, weight loss and impaired coordination of limb movements [3].

People with diabetes continue to increase every year due to the increasing lifestyle of the community. Information from the International Diabetes Federation (IDF) in 2017, there were 425 million adults with diabetes mellitus. Furthermore, it increased in 2019 with the number of sufferers reaching 463 million people. The projected increase in people with diabetes in 2030 will reach 578
million and in 2045 it will reach 700 million [4]. The position of the country of Indonesia with diabetes mellitus sufferers is in the 6th position in the world.

DM can cause chronic complications if not treated quickly and appropriately. Complications that can occur such as eye complications in the form of diabetic retinopathy, cataracts, glaucoma, diabetic neuropathy, diabetic nephropathy, skin infections and stroke[5]. DM treatment takes a long time and therapy using synthetic drugs will cause side effects, therefore the search for herbal-based drugs is the community's choice [6].

Idat plant (Cratoxylum glaucum) is a local plant from Bangka Belitung. Utilization of idat plants on the shoots as a mixture in traditional Bangka cuisine, as traditional medicine is used to reduce high blood pressure. The genus Cratoxylum by the Chinese state community is used as a traditional medicine to treat fever, cough, diarrhea, digestive tract diseases, abdominal pain and can tighten the skin [7,8]. A very intensive study of the bioactivity of the genus Cratoxylum focuses on antidiabetic. Several studies on the genus Cratoxylum from China and Thailand showed a very high inhibitory ability against the inhibition of the α-glucosidase enzyme, protein tyrosine phosphate, and the inhibitory carbohydrate hydrolysis enzyme because it contains phenolicanthraquinone and xanthone compounds[9].

Based on previous research on Cratoxylum glaucum species, it was found that the ethyl acetate extract contained phenol hydroquinone, flavonoid and steroid phytochemicals, with high antioxidant bioactivity [10]. Based on research on the type of phenolic contained in the leaf extract of C. glaucum, anthraquinone and xanthone compounds were found in the extract [11]. Very intensive research on C. glaucum species is needed to optimize the potential of local plants as antidiabetic herbal medicines. Therefore, the importance of more intensive research on C. glaucum species regarding the content of secondary metabolites in C. glaucum stems and their antidiabetic power.

2. Materials and methods
2.1. Material and equipment
The materials used in this study were idat shoots stem extract, ethyl acetate (C₄H₈O₂), chloroform (CHCl₃), FeCl₃, magnesium solids, amyl alcohol (37% HCl and 95% ethanol mixture), Na₂CO₃, distilled water, AlCl₃, NaH₂PO₄ solid, solid p-nitrophenyl-α-D-glucopyranoside (pNPG) and α-glucosidase enzyme solution. Equipment used includes jar, sample bottle, wood shaver, spatula, dropper, volume pipette, beaker, measuring cup, test tube, stirring rod, measuring flask, separating funnel, analytical balance, rotary evaporator (IKA RV 20 Basic).

2.2. Sample Preparation
The stems of idat plants (Cratoxylum glaucum) from Kimak Village, Bangka Regency, were dried in an open room protected from sunlight. The dried samples were ground using a wood shavings into dry powder.

2.3. Extraction
The dry powder of the stems of ±2 kg of idat plants was soaked using ethyl acetate solvent for 1x24 hours with a ratio of 1:10 sample and solvent placed at room temperature. After the immersion, the filtrate was taken from the filtered residue. then the filtrate obtained was concentrated using a rotary evaporator [12].

2.4. Secondary Metabolite Phytochemical Test
Phytochemical tests were carried out qualitatively at the Chemical Laboratory of the Faculty of Fisheries, Agriculture and Biology (FPPB), Bangka Belitung University.

2.4.1. Phenolic Test
The sample was put into a tube dripped with 5% FeCl₃. A positive test indicates the color of the solution becomes dark green [13].
2.4.2 Flavonoid Test
The sample was put into a test tube, 0.1 mg magnesium solids and 0.4 mL amyl alcohol were added (a mixture of 37% HCl and 95% ethanol). Then 4 mL of alcohol was added. The test is positive if the formation of a yellow, red or orange color on the amyl alcohol layer [14].

2.5. Antidiabetic Test
Antidiabetic testing was carried out in vitro at Indonesian Institute of Sciences, Chemistry with the α-glucosidase enzyme inhibition method. Following are the stages of antidiabetic testing [15]:

2.5.1. Material Preparation
3.59 g of Na₂HPO₄ was dissolved in 100 mL of distilled water (solution A) and 1.39 g of NaH₂PO₄ was dissolved in 100 mL of distilled water (solution B). Solution A was added to solution B until it reached pH 7.0, then distilled water was added so that the volume became 200 mL. This was followed by making 150.65 mg of p-nitrophenyl-α-D-glucopyranoside solution dissolved in 25 mL of phosphate buffer pH 7. The substrate solution was diluted to 5 mM. Preparation of 1.0 mg α-glucosidase enzyme solution was dissolved in 100 mL of phosphate buffer pH 7 containing 200 mg of bovine serum albumin. Testing the enzyme stock was diluted 10 times with phosphate buffer pH 7. Making sodium bicarbonate as much as 2.12 grams dissolved in 100 mL of distilled water in a volumetric flask to the limit mark.

Measurement of α-Glucosidase activity inhibition was carried out by mixing 250 μL of 5 mM p-nitrophenyl-α-D-glucopyranoside solution and 495/490 μL of phosphate buffer pH 7 0.1M and added to a test tube containing 5 μL (standard solution)/10 μL (sample solution) in DMSO with various concentrations as above. After the homogeneous solution was incubated for 5 minutes at 37°C, the reaction was started by adding 250 μL of α-glucosidase solution. Incubation was continued for 15 minutes. The reaction was stopped by the addition of 1 ml of 0.2 M Na₂CO₃. Enzyme activity was measured based on the reading of the absorption of p-nitrophenol formed at a wavelength of 400 nm. Quercetin was used as a standard for comparison.

2.5.2. Data Analysis α-glucosidase inhibition test
The absorbance measurement results can be determined by the percent value of α-glucosidase inhibition through calculations using the equation (1)

\[
% \text{Inhibition} = \left( \frac{\text{Blank Absorbance (DMSO)} - \text{Test Sample Absorbance}}{\text{Blank Absorbance (DMSO)}} \right) \times 100\% \quad (1)
\]

Calculation of IC50 by using the linear regression equation \( y = ax + b \). The sample concentration as the x-axis while the % inhibition as the axis. The equation \( y = ax + b \) can be calculated IC50 value using the equation (2) [16].

\[
IC_{50} = \frac{50 - a}{b} \quad (2)
\]

3. Result and Discussion
Idat stem were extracted using the maceration method. Maceration method is the most commonly used method to obtain active compounds contained in samples, and this method is suitable for active compounds that are not heat resistant. The maceration process was carried out using ethyl acetate as solvent.

Phytochemical screening was carried out qualitatively, by observing the changes that occurred in the form of color changes. Phytochemical analysis aims to determine the content of secondary metabolites contained in idat stem samples. This phytochemical test was carried out especially on phenolic compounds and flavonoids which indicated the presence of xanthone and anthraquinone compounds.

Testing of phenolic compounds of ethyl acetate extract showed positive results because a blackish green color was formed. The color change is due to the reaction between the –OH aromatic group in the tannin structure with FeCl₃. The reaction of tannins with FeCl₃ can be seen in Figure 1.
Table 1. Phytochemical Screening Test Results of Idat Stem Extract

| Samples              | Phytochemical    | Reactant | Results                      | Information       |
|----------------------|------------------|----------|------------------------------|-------------------|
| Ethyl Acetate Extract| Phenolic         | FeCl₃    | Dark Green Color Formed      | +                 |
| Flavonoids           | Wilstatur Sianidin|          | Orange Color Formed          | +                 |

![Figure 1. Reaction between tannins and FeCl₃][17].

The flavonoid compound was tested by reacting the ethyl acetate extract with HCl and magnesium metal. The test results showed that there was a change in color to orange, so it was concluded that the extract was positive for flavonoids. Chloride will protonate flavonoids to form flavonoid salts (red) while magnesium metal will reduce flavonoids to form orange color [18]. The formation of a red color is due to the oxygen in the carbonyl group of the flavonoid group compound will be protonated due to the reaction with HCl and form a red flavilium salt. The flavonoid identification reaction can be seen in the following Figure 2.

![Figure 2. Flavonoid test reaction][19]

The antidiabetic activity test was carried out in vitro by inhibiting the α-glucosidase enzyme. The principle of measuring the inhibition of the alpha glucosidase enzyme is that the test sample and the p-nitrophenol-α-D-glucopyranoside (p-NPG) substrate are incubated and reacted with the α-glucosidase...
enzyme. The results of the antidiabetic test of the ethyl acetate extract showed that the extract was able to inhibit the activity of the α-glucosidase enzyme.

Antidiabetic test data showed that the α-glucosidase inhibitor of ethyl acetate extract had very strong activity with an IC$_{50}$ of 4.21 g/mL. Based on the study of antidiabetic bioactivity against the genus *Cratoxylum* in Kissinger’s study [20], it was found that the antidiabetic activity of α-glucosidase methanol extract of irat bark stem (*Cratoxylum arborescens*) had an IC$_{50}$ value of 505.23 g/mL. In another study conducted by Arsakit [9], the antidiabetic activity of the stem extract of *Cratoxylum formosum* subsp. had an IC$_{50}$ of 31.1 g/mL. Therefore, it can be concluded that the ethyl acetate extract of the stem shoots of i dat (*Cratoxylum glaucum*) on antidiabetic activity has a lower IC$_{50}$ value compared to other species, the smaller the IC$_{50}$ value of a test extract, the better its bioactivity as antidiabetic. So it can be concluded that the stem i dat has the potential to be developed as an antidiabetic treatment therapy.

4. Conclusion

The ethyl acetate extract of *Cratoxylum glaucum* stem contains phenolic and flavonoid compounds. The presence of these compounds supports the antidiabetic power of ethyl acetate extract in excellent α-glucosidase inhibition. The IC$_{50}$ value in α-glucosidase inhibition in ethyl acetate extract was 4.21 g/mL. The results of the antidiabetic test showed that the stem i dat has the potential to be developed into an antidiabetic drug.

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