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# Phytochemical Screening and α-glucosidase Inhibitory of Secang Wood (*Caesalpinia sappan* L.)

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**Abstract**
*Secang wood (Caesalpinia sappan* L.*) is a part of the Secang plant which is widely used to treat various diseases, including diabetes. Based on previous research, secang wood contains flavonoids which have the potential to inhibit the α-glucosidase enzyme. The research aims to determine the potential of ethanol extracted by meseration using 96% ethanol. Inhibition of activity was tested using microplate reader which was measured at a wavelength of 405 nm with acarbose as a comparison. The results of the research show that the ethanol extract of secang wood has inhibitory activity, including it in the active category based on the % inhibition value obtained at 83.63%. Meanwhile, acarbose has inhibitory activity, including it in the very active category with a %inhibition value of 79%.*

## I. Introduction

Diabetes mellitus is a disease characterized by increased blood glucose levels caused by a lack of insulin, either absolute or relative. Currently, diabetes mellitus is a serious threat to humans and is the 7th leading cause of death in the world and Indonesia is ranked 4th after the United States, India, and China for the highest number of people with diabetes mellitus in the world with DM incidence from year to year. The prevalence of diabetes mellitus in Indonesia will continue to increase in 2013 by 6.9%, while in 2018 it will be 8.5% (Karim *et al*., 2021) .

There is a therapeutic approach that can be used to treat diabetes mellitus, namely by inhibiting enzymes related to glucose absorption in the body, such as the α-glucosidase enzyme which functions to accelerate glucose absorption by the small intestine by catalyzing the hydrolytic cleavage of oligosaccharides into monosaccharides, which causes an increase in glucose levels. blood in the body after eating to slow or delay the absorption of glucose in the intestine which can prevent an increase in post-prandial blood glucose levels, inhibiting the enzyme α-glucosidase (Karim *et al*., 2021).

Another approach in treating diabetes mellitus is by administering antioxidants, various supplements containing antioxidants and/or factors that can increase the production of nitric oxide (NO) which have the potential to improve endothelial dysfunction and mitochondrial function in cells, as well as reducing the activity of the NAD(P)H enzyme. oxidase. In cases of macrovascular/microvascular complications in diabetes mellitus sufferers, antioxidant therapy is useful if given simultaneously with therapy to control blood pressure, dyslipidemia conditions, and control glucose levels optimally (Prawitasari, 2019).

Antioxidants are compounds that slow down or prevent the oxidation process, whereas according to Hudson BJF (1990), antioxidants are defined as compounds that can prevent oxidation reactions by stopping chain reactions due to the emergence of free radicals.

The discovery of new drugs and the need for new drug preparations continues to increase in line with the demand for improvements in human health standards that can be obtained through the use of more effective and efficient drugs. One way that can be done to achieve this goal is by optimizing the use of medicinal plants that are widely used and have been empirically proven to provide therapeutic effects (Syarif *et al*., 2016).

The sappan plant (*Caesalpinia sappan* L.) is empirically commonly used by the people of the district. Bone, South Sulawesi province as a treatment for diabetes mellitus. The extent of the influence of sappan (*Caesalpinia sappan* L.) in curing diabetes mellitus is not yet known with certainty. Secang wood (*Caesalpinia sappan* L.) has brazilin compounds which give a red color which is included in the flavonoid group as isoflvanoid which is an antioxidant compound (Yusuf *et al*., 2019).

## II. Research Method

### II.1 Tools and Materials

The tools used are a stir bar, maceration vessel, blender (Philips®), porcelain cup, Buchner funnel, ELISA reader (Biotek®), watch glass, beaker (Phyrex®), measuring cup (Phyrex®), measuring flask (Phyrex ®), refrigerator, tweezers, microliter pipette (Eppendorf®), drop pipette (Pyrex®), volume pipette (Phyrex®), knife, horn spoon, analytical balance (Ohaous®), vial, pan and water bath. Meanwhile, the materials used are acarbose, distilled water, α-Glucosidase derived from recombinant Saccharomyces cerevisiae (Sigma Aldrich, USA), 96% ethanol, phosphate buffer solution pH 7, $Na_2CO_3$ solution (Sigmaaldrich, USA), p-nitrophenyl-α- substrate. Dglucopyranoside (PNPG) (Wako Pure Chemical Industries, Ltd., Japan) and Secang wood (*Caesalpinia ssappan* L.) were extracted using the maceration method.

### II.2 Research Procedure

#### II.2.1 Preparation of Solution Materials

The procedure for preparing test materials is based on research from Maryam *et al* (2020) which has been modified.

**Sodium carbonat solution ($Na_2CO_3$) 200 mM.** $Na_2CO_3$ 5.3 g was weighed and then dissolved in 250 mL of CO2-free water until a concentration of 200 mM was obtained.

**Acarbose Solution.** 1 mg of acarbose was weighed and dissolved in 100 mL of pH 7 phosphate buffer. A solution with a concentration of 10 ppm was obtained. After that, it was made in five concentration variations, namely 0.2 ppm, 0.4 ppm, 0.6 ppm, 0.8 ppm and 1 ppm.

**Secang wood extract solution.** 10 mg of the extract was weighed and dissolved in 10 mL of pH 7 phosphate buffer until homogeneous. A stock solution with a concentration of 1000 ppm was obtained. After that, it was made in five concentration variations, namely 100, 125, 150, 175 and 200 ppm.

**Substrate Solution *p*-nitophenyl-α-Dglucopyranoside (PNPG) 5 mM.** The substrate solution was made by dissolving 15.062 mg of *p*-nitophenyl-α-D-glucopyranoside (PNPG) and the volume was increased with aqua demineralisate to 10 mL to obtain a concentration of 5 mM.

**α-glucosidase Enzyme Solution Master Solution.** The α-glucosidase enzyme was weighed as much as 1 mg and dissolved in 100 mL of pH 7 phosphate buffer (in each mg there was 28 U). Enzyme Solution 0.25 U/mL. The stock solution was pipetted at 0.089 mL and the volume was made up to 10 mL with pH 7 phosphate buffer.

#### II.2.2 α-glucosidase Enzyme Activity Inhibition Test

**Blanko Test.** 36 µL of pH 7 phosphate buffer and 17 µL of 5 mM *p*-nitrophenyl-α-D-glucopyranoside (PNPG) substrate were put into the well and incubated in a water bath for 5 minutes at 37°C. After the incubation period was complete, 17 µL of α-glucosidase enzyme was added to the well and incubated again in the water bath for 15 minutes at 37°C. After the second incubation period was complete, 100 µL of 200 mM $Na_2CO_3$ was added to stop the reaction and the absorbance was measured using an ELISA reader at a wavelength of 405 nm.

#### II.2.3 Blanko Control Testing

36 µL of pH 7 phosphate buffer and 17 µL of 5 mM *p*-nitrophenyl-α-D-glucopyranoside (PNPG) substrate were put into the well, then incubated in a water bath for 20 minutes at 37°C. After the incubation period was complete, 200 mM $Na_2CO_3$ was added as much as 100 µL for the reaction. The absorbance was measured using an ELISA reader at a wavelength of 405 nm. Comparative Test (Acarbose) 36 µL of pH 7 phosphate buffer was put into the well, then 30 µL of the comparator with a concentration of 0.2 ppm was put into the well, likewise for the comparator with a concentration of 0.4 ppm, 0.6 ppm, 0.8 ppm, and 1 ppm. 5 mM *p*-nitrophenyl-α-D-glucopyranoside (PNPG) substrate was added as much as 17 µL and incubated in a water bath for 5 minutes at 37°C, after the incubation period was complete the α-glucosidase enzyme was added as much as 17 µL to each well and incubated again in a water bath for 15 minutes at 37°C. After the incubation period was complete, 100 µL of 200 mM $Na_2CO_3$ was added to stop the reaction. The absorbance of the sample was then measured using an ELISA reader at a wavelength of 405 nm.

#### II.2.4 Comparative Control Testing

36 µL of pH 7 phosphate buffer was put into the well, then 30 µL of the reference with a concentration of 0.2 ppm was put into the well, likewise for the comparison with concentrations of 0.4 ppm, 0.6 ppm, 0.8 ppm and 1 ppm. 5 mM *p*-nitrophenyl-α-D-glucopyranoside (PNPG) substrate was added as much as 17 µL and incubated in a water bath for 20 minutes at 37°C. After the incubation period was complete, 100 µL of 200 mM $Na_2CO_3$ was added to stop the reaction. The absorbance of the sample was then measured using an ELISA reader at a wavelength of 405 nm (Ahmad *et al*, 2023).

#### II.2.5 Sample Testing (Ethanol Extract of Secang Wood)

36 µL of pH 7 phosphate buffer was put into the well, then 30 µL of roasted secang woodseed ethanol extract with a concentration of 100 ppm was put into the well, likewise for Secang wood ethanol extract with concentrations of 125 ppm, 150 ppm, 175 ppm and 200 ppm. 5 mM *p*-nitrophenyl-α-Dglucopyranoside (PNPG) substrate was added as much as 17 µL and incubated in the water bath for 5 minutes at 37°C, after the incubation period was

complete, the enzyme α-glucosidase was added as much as 17 µL to each well and incubated again in the water bath for 15 minutes at 37ºC. After the incubation period was complete, 100 µL of 200 mM $Na_2CO_3$ was added to stop the reaction. The absorbance of the sample was then measured using an ELISA reader at a wavelength of 405 nm.

### II.2.6 Sample Control Testing

36 µL of pH 7 phosphate buffer was put into the well, then 30 µL of roasted secang woodseed ethanol extract with a concentration of 100 ppm was put into the well, likewise for secang wood ethanol extract with concentrations of 125 ppm, 150 ppm, 175 ppm and 200 ppm. 5 mM *p*-nitrophenyl-α-Dglucopyranoside (PNPG) substrate was added as much as 17 µL and incubated in a water bath for 20 minutes at 37°C, after the incubation period was complete, 200 mM $Na_2CO_3$ was added as much as 100 µL to stop the reaction. The absorbance of the sample was then measured using an ELISA reader at a wavelength of 405 nm.

## III. Results and Discussion

Secang (*Caesalpinia sappan* L.) has high antioxidant activity. The red dye found in Secang (*Caesalpinia sappan* L.) is known as a brazilin group compound, which is an antioxidant compound that has catechol in its chemical structure and can protect the body from poisoning due to free radicals.

The sample used in this research was ethanol extract of sappan wood and acarbosa as a comparison. acarbose is an oligosaccharide obtained from the fermentation process of Actinoplanes uthahensis which works to inhibit the enzyme αglucosidase which is located in the wall of the small intestine. This extract was obtained through extraction using maceration using 96% ethanol solvent. The maceration method is used because the flavonoid content is not resistant to high temperatures and the process does not occur heating like other methods, so it is hoped that the antioxidant content contained in the extract stream is not damaged (Ahmad *et al*, 2023).

Alpha-glucosidase is the key enzyme responsible for the breaking of oligosaccharides and disaccharides into monosaccharides suitable for absorption. Inhibition of alpha-glucosidase is one of the main strategies to counteract the metabolic changes associated with hyperglycemia and type 2 diabetes (Zhang *et al*., 2019)

This research uses acarbose as a standard which is an oligosaccharide obtained from the fermentation process of Actinoplanes uthahensis which works to inhibit the enzyme αglucosidase which is located in the wall of the small intestine. The substrate *p*-nitrophenyl-α-Dglucopyranoside is also used as a model to represent carbohydrates in the body, where the enzyme will break down the substrate into glucose and *p*-nitrophenol. In accordance with the principle of this test, namely measuring enzyme activity based on the absorbance results of pnitrophenol which is the result of hydrolysis of the substrate *p*-nitrophenyl-α-D-glucopyranoside (PNPG). The higher the ability of plant components to inhibit the α-glucosidase enzyme, the smaller the p-nitrophenol product formed, which is indicated by a color change on the substrate, namely a fading yellow color. It is known that enzymes are proteins that are thermolabile so that in their processing, the temperature and pH must be maintained in an optimum state. The temperature used was 37ºC and pH 7, therefore pH 7 phosphate buffer was used as a solvent. The microplate reader instrument was used and measured at a wavelength of 405 nm.

The TLC profile (Figure 1) of ethanol extract of secang wood by TLC using and mobile phase n-hexane eluent: ethyl acetate (1:4). The spots were observed using a UV 254 nm and 366 nm. Idnetifikation of the compounds by spraying with various specific reagents were positive for flavonoids, alkaloids, and phenols (Figure 2). Then for the calculation results of the Rf values at spots 1, 2, 3, and 4, the values obtained were 0.872, 0.690, 0.52, and 0.327, respectively (Table 1). The inhibition of the α-glucosidase enzyme at respective concentrations of 100ppm, 125ppm, 150ppm and 200 ppm, the inhibition percentages were 49.57%, 60.03%, 68.25%, 75.61% and 83.63%, respectively (Table 2). According to the results, it shows that the ethanol extract of secang wood has an activity in inhibiting the α-glucosidase enzyme. This activity was predicted due to the chemical compounds that contain in secang wood which especially phenolic groups.

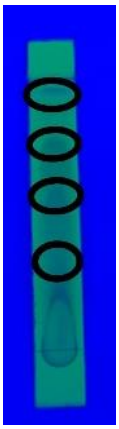

**Figure 1**. TLC Profile of secang wood extract

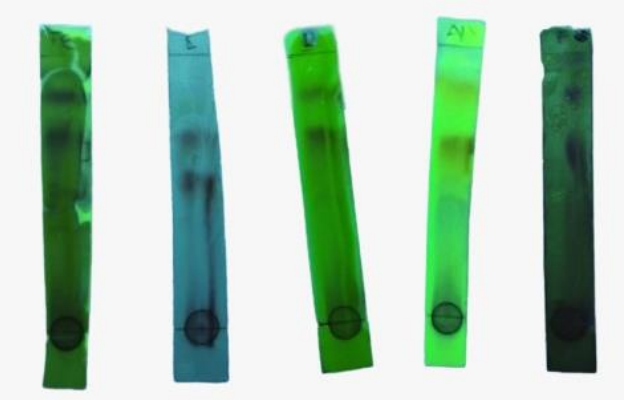

**Figure 2**. Phytochemical profile (visualization: $FeCl_3$, Lieberman Burchard, Dragendorff, $AlCl_3$ and Folin Ciocalteu, respectively)

**Table 1**. The Rf data of TLC profile

| UV light | Spot Number | Rf |
|---|---|---|
| 254 nm | 1 | 0.872 |
| | 2 | 0.69 |
| | 3 | 0.52 |
| | 4 | 0.327 |

**Table 2.** The data of inhibitory α-glukooksidase of secang extract

| Title | Concentration | Absorbance | % Inhibition |
|---|---|---|---|
| Acarbose | 1 | 0.969 | 96.9 |
| | 0.8 | 0.938 | 93.8 |
| | 0.6 | 0.899 | 89.9 |
| | 0.4 | 0.856 | 85.6 |
| | 0.2 | 0.79 | 79 |
| Secang extract | 200 | 0.836 | 83.6 |
| | 175 | 0.756 | 75.6 |
| | 150 | 0.682 | 68.2 |
| | 125 | 0.6 | 60 |
| | 100 | 0.495 | 49.5 |

## IV. Conclusions

The TLC results showed that the ethanol extract of Sappan wood contains alkaloid, flavonoid and phenol compounds. Regarding the inhibition of the α-glucooxidase enzyme. Based on the results of the research conducted, it can be concluded that the ethanol extract of secang wood (*Caesallpinia sappan* L.) has inhibitory activity in the active category with an inhibition percentage of 83.6% at a concentration of 200 ppm.

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