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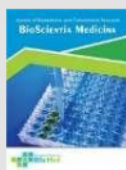
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Inhibitory Effect of N-Hexane Fraction of Cherry Parasite (*Dendrophthoe pentandra* L.) on Alpha-glucosidase

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ABSTRACT

Background. The cherry parasite (*Dendrophthoe pentandra* L.) is a semi-parasitic plant that takes food from the host body so that phytochemical is the same as the host plant. These phytochemicals have an effect on inhibiting the action of the enzyme alpha-glucosidase, such as flavonoids, saponins, tannins, alkaloids, and terpenoids/steroids. This study aims to find out the effect of the cherry parasite on inhibiting the enzyme alpha-glucosidase. **Methods:** This is an in vitro experimental study. The cherry parasite was fractionated with n-hexane and reacted by the enzyme. Acarbose was used as a positive control. The inhibitory effect on the alpha-glucosidase was determined from the IC50 value by measuring the absorbance of p-nitrophenol using spectrophotometry as a result of the reaction of the enzyme. Screening phytochemical to determine the phytochemical of n-hexane fraction. This research was conducted at the Laboratory of Biochemistry and Medical Chemistry, Faculty of Medicine, Universitas Sriwijaya, Palembang. **Results:** The yield of the n-hexane fraction was 7%. A phytochemical test showed that the sample contain flavonoids and terpenoids. The IC50 value of n-hexane fraction was 106,333 ppm and categorized not active. **Conclusion:** The n-hexane fraction of the cherry parasite contains flavonoids and terpenoids that are considered not active in inhibiting the action of the enzyme alpha-glucosidase.

4. 1. Introduction

Traditional medicine has been around for a long time and is considered a tradition in certain areas. One of the plants that have been studied and have benefits for treatment is the cherry tree (*Muntingia calabura*). Almost all parts of the cherry tree have medicinal benefits, cherry fruit and leaves (*Muntingia calabura* L.) can be used as antidiabetic because the phytochemical can inhibit the action of the α -glucosidase enzyme. Cherry leaves contain alkaloids, flavonoids, saponins, phenolic, tannins, terpenoids.¹⁻³

The part of the cherry tree which is also thought to have the same benefits as antidiabetics is the cherry parasite (*Dendrophthoe pentandra* L.). Cherry parasites are semi-parasitic plants that take food from their host

bodies and contain the same active compounds as their host plants.⁴⁻⁵ Phytochemical screening of ethanol extracts of cherry parasite shows the results that cherry parasite (*Dendrophthoe pentandra* L.) contains flavonoids, alkaloids, terpenoids, tannins, and saponins.⁵ That phytochemicals have biological effects such as antidiabetic, antiproliferative, and apoptosis against cancer cells.⁵⁻⁷

The phytochemicals such as flavonoids, saponins, tannins, and terpenoids have a mechanism to inhibit the enzyme α -glucosidase. Flavonoids have an effect on inhibiting the enzyme α -glucosidase through hydroxylation bonds and substitution in the β

Terpenoids have rings that can bind to the α -glucosidase enzyme. Saponins act as inhibitors of α -glucosidase enzymes by damaging the composition of cell membranes, while tannins as astringents can preserve protein in the intestinal mucous membrane and form a layer that protects the intestine, thereby inhibiting glucose absorption.⁷⁻⁹

This study uses n-hexane as a solvent because n-hexane is a non-polar organic solvent that is inert or stable so that it can easily attract compounds contained in cherry parasites, especially non-polar compounds.¹⁰ Meanwhile there is no research about the effect of the n-hexane fraction of cherry parasites on the α -glucosidase enzyme. Therefore, this study aims to determine the phytochemicals and effects of the n-hexane fraction of cherry parasites (*Dendrophthoe pentandra* L.) to inhibit the action of enzyme α -glucosidase.

2. Methods

This is an experimental study that used spectrophotometry as a method to determine the ability of the phytochemical of the n-hexane fraction of cherry parasite (*Dendrophthoe pentandra* L.) to inhibit the action of the α -glucosidase enzyme. This research was conducted at the Biochemistry Laboratory, Faculty of Medicine, Universitas Sriwijaya, Palembang.

Cherry parasite leaves (*Dendrophthoe pentandra* L.) were obtained from Palembang City. The leaves were identified in accordance with the morphology and characteristics of the leaves and according to biologists and literature, there is a cherry parasite leaves (*Dendrophthoe pentandra* L.) of the genus *Dendrophthoe*.

The tools that used in this research are blender (Philips), analytical balance (ABS), UV-Vis spectrophotometry (Shimadzu), pH meter (ATC®), micro pipette (Eppendorf®), measuring flask (IWAKI®), measuring cup (IWAKI®), and filter paper.

The chemicals used are ethanol (Bratachem), n-hexane (Bratachem), aquades (Bratachem), α -glucosidase enzyme (Sigma Aldrich), p-nitrophenyl- α -D-glucopyranoside (Sigma Aldrich), buffer phosphate

pH 6,8 (Certipur®), DMSO (Merck), BSA (ROFA), acarbose (Glucobay®).

Two grams of simplisia powder of cherry parasites leaves were macerated in ethanol for 3x24 hours. Then the results were filtered by using filter paper. The filtrate was collected and evaporated in an oven to give thick liquid materials of ethanol extract. Next, the ethanol extract as much as 30 grams was subsequently fractionated with n-hexane. Shake the extract until the gas disappears and then let stand for approximately 10-30 minutes so that it reaches the saturation condition which is marked by the formation of two layers, a layer of water and n-hexane.

Phytochemical screening used qualitative methods established by Harborne.¹¹ The phytochemical test were identified flavonoids, saponins, terpenoids/steroids, alkaloids, and tannins. Flavonoid identification was carried out by reacting 2 mL of n-hexane fraction of cherry parasite leaves with 0,5 mg of Magnesium powder and 2 mL of 2N HCl. Positive results if red or yellow are formed.

Identification of saponin by adding distilled water to the sample with a ratio of 1: 1 between distilled water and extract, then shaken vertically for 10 seconds. If foam 1-10 cm arises for about 10 minutes, the results show positive saponins.

Identification of terpenoids is done by dissolving the fraction with 0.5 mL chloroform, then adding 0.5 mL acetic acid anhydride, and continuing with the addition of 2 mL concentrated sulfuric acid through the tube wall. If a brownish or violet ring is formed at the border of the solution, it indicates a triterpenoid and a greenish-blue ring indicates an asteroid.

While the identification of alkaloids is done by adding 10 mL chloroform to the fraction solution and adding a few drops of ammonia. Then suction the chloroform fraction with a dropper. After that, acidize it with H₂SO₄ 2M. The results are taken and reacted with Meyer, Dragendorff, and Wagner reagents. Positive results if white sediments form on Meyer's erection, orange-red deposits on Dragendorff, and brown deposits on Wagner. Positive alkaloids if positive all three. Tannin identification was carried out by reacting

1 mL of fraction solution with 10% FeCl₃. Positive if dark blue or greenish-black is formed.

Alpha glucosidase enzyme inhibiting activity test using the established methods by Loranza,¹¹ which has been modified. A total of 10 µL of the sample (100 ppm, 50 ppm, 25 ppm, and 6,25 ppm) was mixed with 490 µL of phosphate buffer pH 6.8 and 250 µL of 10mM p-nitrophenyl-α-D-glucopyranoside substrate solution. Incubate for 5 minutes at 37°C. After that, added to 250 mL of an enzyme solution 0,15 U/mL, incubated at

37°C for 15 minutes. To stop the reaction process, add Na₂CO₃ 0,2 M as much as 1000 µL. Next, measure the absorbance value at λ 400 nm.

Acarbose was used as positive control and carried out as in the sample solution. And the blanko is made by replacing the n-hexane fraction with DMSO. Every single solution was made a control solution to determine the work of the enzyme. The working procedure on this research can be seen in table 1.

Table 1. Enzyme inhibiting activity test procedure

Reagen	Volume (µL)					
	B ₀	B ₁	S ₀	S ₁	A ₀	A ₁
n-Hexane fraction	-	-	10	10	-	-
Acarbose	-	-	-	-	10	10
DMSO	10	10	-	-	-	-
Phosphate Buffer pH 6,8	490	490	490	490	490	490
Substrat p-NPG	250	250	250	250	250	250
Incubate at 37°C for 5 minutes						
α-glucosidase enzyme	-	250	-	250	-	250
Na ₂ CO ₃ 0,2 M	1000	-	1000	-	1000	-
Incubate at 37°C for 15 minutes						
α-glucosidase enzyme	250	-	250	-	250	-
Na ₂ CO ₃ 0,2 M	-	1000	-	1000	-	1000
Measure the absorbance at λ 400 nm						

B₀ = Blanko control, B₁ = Blanko, S₀ = Sample control, S₁ = Sample, A₀ = Acarbose control, A₁ = Acarbose.

Data from this study were obtained from the absorbance value of the sample and control measured by using a spectrophotometer. From the absorbance value will be obtained percent inhibition. The α-glucosidase enzyme inhibiting activity can be expressed in IC₅₀ values obtained from the linear regression equation, the concentration of the sample as the x-axis, and percentage inhibition as the y-axis.

3. Results

Extraction, fractionation, and phytochemical test

From two grams of Simplicia cherry parasite leaves, obtained the ethanol extract 33,3 grams and the extract yield is 5.5%. While the results of the n-hexane fraction were 2,1 gram and the yield of the fraction was 7%. The n-hexane fraction of cherry parasites contains flavonoids and terpenoids (table 2).

Table 2. Phytochemical test results of n-hexane fraction

Phytochemical	Result
Flavonoids	Positive
Terpenoids	Positive
Tannins	Negatif
Saponins	Negatif
Alkaloids	Negatif

Alpha-glucosidase inhibition test

Alpha-glucosidase inhibition test was analyzed using the IC₅₀ values. The IC₅₀ value is the effectiveness of the substance which can inhibit 50% of the enzyme

activity. In this research, the positive control was acarbose which was made and arranged the same as the sample. In addition, a blank solution was also made to obtain the percent of inhibition value. The n-hexane

fraction, acarbose, and blank solution were measured using spectrophotometry with a wavelength of 400 nm

to determine the absorbance value.

Table 3. Alpha-glucosidase inhibition

Sample	IC ₅₀ (ppm)
n-hexane fraction	2. 106,333
Acarbose	4. 20,213

The absorbance value is used to determine the percent of inhibition value. The highest percentage of inhibition value of n-hexane fraction was at a concentration of 100 ppm and the lowest at a concentration of 6.25 ppm. Meanwhile, in acarbose, the highest percentage inhibition value in concentration was 12.5 ppm.

IC₅₀ was obtained from the linear regression between the percentage of inhibition and the concentration of the fraction n-hexane cherry parasites. The result showed that IC₅₀ values of fraction n-hexane cherry parasites are 106,333 ppm. It is mean the fraction n-hexane of cherry parasites inactive as an antidiabetic. Meanwhile, in acarbose, the IC₅₀ values is 20,231 ppm which means activated as an antidiabetic (table 3).

4. Discussion

A phytochemical test was carried out to determine the secondary metabolic compounds contained in the fraction n-hexane of cherry parasites. The test is carried out to qualitatively fraction n-hexane of cherry parasites by mixing the sample with the reagent from each phytochemical test to produce a chemical reaction that is characterized by color-changing of fraction according to the compound. In this study, five compounds were tested, flavonoids, terpenoids/steroids, tannins, saponins, and alkaloids.

The n-hexane fraction of the cherry parasite contains flavonoids and terpenoids. It is because the n-hexane fraction is a non-polar compound so the polar compounds such as tannins, saponins, and alkaloids cannot be attracted by the n-hexane fraction which has a different polarity level. Terpenoids are non-polar compounds so they can easily be attracted by non-

polar compounds as well as n-hexane. While flavonoids which are divided into several types have different levels of polarity. Types of aglycon flavonoids such as isoflavones, flavanones, flavones, and flavonoids that are methoxylated have non-polar properties so that they can only be attracted to non-polar solvents, such as n-hexane solvents.^{11,13} Therefore, in this study the flavonoids contained in the n-hexane fraction of the cherry parasites leave maybe the type of non-polar flavonoids such as isoflavones, flavanones, flavones, and flavonoids. The phytochemicals of parasite leaves depend on the phytochemical present in their host and are also influenced by the solvent used. Usually, the phytochemicals have the same level of polarity as the solvent.¹³

Alpha-glucosidase inhibition test

The α -glucosidase enzyme works by breaking down carbohydrates in the form of oligosaccharides and polysaccharides into monosaccharides in the intestinal wall. In addition, the α -glucosidase enzyme works by hydrolyzing p-nitrophenyl- α -D-glucopyranoside into p-nitrophenol and α -D- glucose.¹⁴ The activity of these enzymes can be inhibited by acarbose and the fraction so the result can not be formed. The results showed that the n-hexane fraction had an inhibitory effect on enzymes, seen from the low levels of the formed p-nitrophenol product which the absorbance value was small. The percent value of inhibition was different for each concentration.

The IC₅₀ value between the n-hexane fraction and acarbose is very far away. This is because the acarbose has been validated that can inhibit the action of the α -glucosidase enzyme. The antidiabetic nature of IC₅₀ is divided into several groups: very active (<11

ppm), active (11-100 ppm), and inactive (> 100 ppm).¹⁵ Thus, it can be seen that the IC₅₀ value of the n-hexane fraction is not active and the acarbose is classified as active as antidiabetic. The acarbose has been clinically proven and based on existing literature can effectively inhibit the action of the α -glucosidase enzyme and has been used as an antidiabetic drug that works by occupying the active side of the α -glucosidase enzyme.

The value of IC₅₀ is influenced by solvents and host plants. Research conducted by Artanti et al.⁴, IC₅₀ value of methanol extract of the parasitic tea leaves was 17,6 ppm and classified as active. Methanol is a polar solvent so it will attract polar compounds as well as quercetin type flavonoids which have been proven to be very active in inhibiting the action of the α -glucosidase enzyme. Meanwhile in the n-hexane fraction of the parasite leaves the type of flavonoid is not polar because of the inability of the fraction to attract polar compounds. In addition, phytochemicals on parasite leaves are influenced by phytochemicals that exist in host plants.^{4,5}

Flavonoids are present in plants as a mixture, rarely found in a single form so there may be several types of flavonoids in one plant. Types of flavonoids that can be inhibiting the action of α -glucosidase enzymes are quercetin, anthocyanidin, isoflavones, and flavanol.^{4,11,12,16} Flavonoids have an effect to inhibit the enzyme α -glucosidase through hydroxylation bonds and substitution in the β ring. While terpenoid has been shown to have activity in inhibiting the enzyme α -glucosidase to form a bond between the ring terpenoids and enzymes so that the process of the breakdown of carbohydrates is disturbed.^{9,17,18} Meanwhile, the n-hexane fraction of cherry parasite leaves does not contain saponins which can inhibit the action of the α -glucosidase enzyme better than other compounds by damaging the composition of the cell membranes.¹⁹ The n-hexane fraction of cherry parasite leaves cannot be considered active in inhibiting the action of the α -glucosidase enzyme even though it has the ability to inhibit the work of the enzyme. Thus, the n-hexane fraction of cherry parasite leaves is considered inactive as antidiabetic.

5. Conclusion

According to the result of this study, the n-hexane fraction of the cherry parasite (*Dendrophthoe pentandra* L.) contains flavonoids and terpenoids which can inhibit the action of the enzyme α -glucosidase but cannot be considered active as antidiabetic.

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