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The Potential of Ethanol Extract of The Yellow Root (*Arcangelisia Flava L*) As The Inhibitor of Enzyme *A-Glucosidase*

Raudhatul Fadhilah[1], and Dedeh Kurniasih[1]
[1]*Department of Chemistry Education, Muhammadiyah Pontianak University, Indonesia*
Corresponding Author: * *Raudhatul Fadhilah[1]*

Abstract :- *The high prevalence of diabetes mellitus requires exploration on medicinal plants that can be used as an antidiabetic. Yellow root plant is one of the plants that are widely spread in Borneo which is used by people to treat diabetes. This study aims to determine the inhibition activity of the yellow root plant in inhibiting α-glucosidase enzyme that is believed to play an important role in the absorption of blood glucose. The results showed that the yellow root extracts containing bioactive compounds of flavonoids, alkaloids, very weakly inhibit the enzyme activity with the IC_{50} value is above 200 ppm. Allegedly the mechanism of yellow root stem as an antidiabetic not only by inhibiting the activity of the α-glucosidase enzyme, but also by stimulating the pancreatic β cells, thereby increasing insulin production or secretion*

Keywords: *diabetes mellitus, the α-glucosidase enzyme, yellow root stem*

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I. Introduction

Diabetes mellitus (DM) is a chronic disease characterized by high blood glucose levels due to the condition where the pancreas does not produce enough insulin or when the body cannot effectively use the insulin which has been produced [1]. The data from World Health Organization (WHO) in 2011 showed that 346 million people worldwide have DM. New approaches to diagnose, treat, early-detection and disease prevention is needed to address this challenge.

There is no cure for DM until now. DM treatment is done by controlling the blood glucose levels through the consumption of synthetic drugs. The use of synthetic drugs as an antidiabetic is likely to have undesirable side effects because of its consumption in a relatively long time[2], one of which resulted in impaired renal function. According Dharmeizar (2015) [3], DM disease accounted for 25% of the causes of chronic blood renal failure.

One way to control blood sugar levels in DM patients is by inhibit the activity of the enzyme α-glucosidase[4]. This enzyme plays as a key at the end of the breakdown of carbohydrates. The α-glucosidase enzyme is a type of hydrolase enzyme that catalyze hydrolysis reaction of the non-reducing terminal of substrates which produce α-glucose[5]. The α-glucosidase enzyme (E.C.3.2.1.20) plays a role in the starch metabolism and glycogen inside the plants and animals tissues, characterized by a variety of substrates such as maltose, glucose amylose, sucrose, etc. [6]

Inhibition on the α-glucosidase enzyme causes inhibition of glucose absorption. Compounds that can inhibit α-glucosidase enzyme called α-glucosidase inhibitors (AGI). AGI compound widely used for the treatment of type 2 diabetes patients [7]. These drugs work competitively in the alimentary canal that can slow down the glucose absorption so it can reduce hyperglycemia after meal.

One of the plants potentially act as inhibitors of the α-glucosidase enzyme is the yellow root (*Archanglisis flava L*). The yellow root is a plant widely spread in Borneo which is used by local people to treat diabetes mellitus or DM [8]. Scientific information regarding yellow root potentials as α-glucosidase enzyme inhibitors has never been reported. Therefore, this study aims to determine the inhibitory activity of the yellow root as the inhibitor of α-glucosidase enzyme.

Experimental

II. Material and Methods

The tools used in this study is a UV spectrophotometer, ELISA reader (Epoch Biotek), a micro pipette, rotary evaporator, oven, grinder, water bath, and a set of glasses.

The materials used in this study is yellow root stem derived from the forest in the Sadaniang District, ethanol 20%, distilled water, α- glucosidase enzyme, p-nitrophenyl-α-D-glucopyranoside (p-NPG), a phosphate buffer

solution of pH 7, bovine albumin serum, acarbose, dimethylsulfoxide (DMSO), HCl 2 N and Na_2CO_3 and materials used for the phytochemical test. All chemicals purchased from Sigma.

III. WORK PROCEDURES

3.1.1 Producing the Ethanol Extract of Yellow Root Stem

The bark of yellow root stem in powder as much as 50 g macerated with ethanol 20% for 1 × 24 hours (twice), filtered through filter paper and evaporated using vacuum rotary evaporator to separate the extract with the solvent. Concentrated extract obtained is made from 6 series of concentration, namely: ppm, 12.5 ppm, 25 ppm and 50 ppm to determine the activity of α-glucosidase inhibitor.

3.2.2 Phytochemicals Test (Harborne 1987)

Identification of Alkaloids. A total of 0.05 grams of yellow root bark extract was added with 10 mL of chloroform and a few drops of ammonia. Chloroform fraction was separated and acidified with H_2SO_4 2 M. Acid fractions were taken and divided into 3 parts, then added Dragendorf, Meyer, and Wagner reagent. The presence of alkaloids indicated by the formation of a white precipitate in Meyer reagent, the red precipitate in Dragendorf reagent, and a brown precipitate in the Wagner reagent.

Identification of Flavonoids. A total of 0.05 grams of yellow root bark extract was added with 10 ml of water. The mixture was then heated for 5 minutes, filtered, and the filtrate was taken. The filtrate was then mixture with Mg powder, 1 mL of concentrated HCl, and 1 ml of amyl alcohol. The mixture is shaken vigorously. Flavonoids positive test is indicated by the appearance of red, yellow, or orange in the lining of amyl alcohol.

Identification of Saponins. A total of 0.05 grams of the yellow root bark extract was added with water and then boiled for a few minutes. The solution was filtered and the filtrate was shaken vigorously. The emergence of a stable froth for 10 minutes after being shaken indicates the presence of saponins.

Identification of Tannins. A total of 0.05 grams of the yellow root bark extract was added with water and then boiled for a few minutes. This solution is filtered and the filtrate was added with FeCl3 1% (b/v). Dark blue or greenish black color showed the presence of tannins.

Identification of Triterpenoids and Steroids. A total of 0.05 grams of the yellow root bark extract was added with 25 mL of ethanol 30% and then heated for 5 minutes and filtered. The filtrate was evaporated and then added with ether. Ether then mixed with Lieberman Buchard reagent. Red or purple color indicates triterpenoids. Green or blue color indicates steroids.

3.2.3 Test of α-Glucosidase Activity through In Vitro

Producing the 4-nitrophenol Standard Curve. Standard curve was constructed using the standard seven-point series, i.e. 15 μM, 30 μM, 45 μM, 60 μM, 75 μM, and 90 μM. Manufacture of standard solution was by dissolving 4-nitrophenol in phosphate buffer solution of pH 7 then divided into 6 concentrations as above. Phosphate buffer of pH 7 is also used as a blank. Furthermore, the absorbance of standard and blank solutions was measured at a wavelength of 400 nm. This experiment was done 3 times.

Test of α-Glucosidase Inhibition

Tests on the inhibition of α-glucosidase enzyme activity using the p-nitrophenyl-α-D-glucopyranoside (p-NPG) substrate and α- glucosidase enzyme. Enzyme solution was made by dissolving 1.0 mg of α-glucosidase enzyme into phosphate buffer solution (pH 7) containing 200 mg of bovine albumin serum, enzyme diluted 25 times with phosphate buffer of pH 7 before use.

Each sample of yellow root bark extracts dissolved into DMSO creating upto 7 series of concentration, i.e. 0, 5, 10, 25, 50, 100, 200 ppm. DMSO used as a correction to the extract absorbance. Cessation of enzyme substrate reaction was done by adding 200 mM of Na_2CO_3. The solution absorbance was then measured using a microplate reader at a wavelength of 400 nm. This experiment was done 3 times.

Acarbose tablet (Glukobay) was used as a positive control. Acarbose dissolved into the buffer and HCl 2 N (1: 1) at a concentration of 1% (b/v) and then centrifuged. As many as 1 mL of the supernatant was prepared and dropped into the reaction mixture as in the extract sample.

IV. Results and Discussion

Test of Phytochemicals

Phytochemical test conducted as a preliminary analysis to identify the presence of a compound without specifying its levels [9]. This test was conducted to identify the existence of an active compound presumably to have anti-diabetic effects. Phytochemical test in this study conducted on the ethanol extract of the yellow root stem using the analysis techniques of color visualization. Phytochemical test results are shown in Table 1.

Table 1. Test Phytochemicals Yellow Root Extract Ethanol

Samples	Parameter Phytochemicals		Results
Yellow root	Alkaloid	Wagner	Positive
		Mayer	Positive
		Dragendorf	Positive
	Steroids		Positive
	Flavonoids		Positive
	Tanin		Positive
	Saponin		Positive
	Triterpenoid		Negative
	Quinone		Positive

Bioactive compounds from several species of herbs are reported to have biological activity which are useful in the treatment of DM through the α-glucosidase enzyme inhibition. Yin Z et al. (2014) [10] mentions that the rhizome extracts containing bioactive compounds terpenoids, alkaloids, phenols, steroids, quinones, showed strong inhibition ability. Alfarabi (2010) [11] also reported that red betel leaves (*Piper crocatum*) containing bioactive phenols, flavonoids, alkaloids, and triterpenoids were able to inhibit the activity of α-glucosidase. In addition, Sugiwati (2005) [12] mentions that the god's crown leaves (*Phaleria macrocarpa* (Scheff.) Boerl) or widely known as *daun mahkota dewa* which contains phenolic compounds, tannins, flavonoids and alkaloids are capable of inhibiting the α-glucosidase enzyme. Based on some of the results of these studies and the test results from yellow root bark extract in this study, therefore the bioactive component of the yellow root stems that have antidiabetic activity through inhibition of the α- glucosidase enzyme in this study are flavonoids, alkaloids, tannins, and quinones.

Inhibition Activity of Yellow Root Ethanol Extracts

Analysis of inhibition power of the yellow root stem ethanol extract towards the α-glucosidase enzyme was done by varying the extract concentrations. Analysis at various concentrations was designed to determine the effect of adding concentration towards the escalation of inhibition power. The analysis results of inhibition power of the yellow roots stem ethanol extract are shown in Table 2, which shows there is an increase in inhibition power in line with the increased concentration of the extract. Linear relationship between inhibition power and concentration of the extract is indicated by the decline in absorbance measured after the addition of the extract at various concentrations.

Table 2. Inhibition power of yellow root ethanol extract towards α-glucosidase activity

[Yellow root stem] (ppm)	Absorbance	Inhibition Power (%)
0	0.862 ± 0.018	-
5	0.811 ± 0.016	4.339 ± 0.773
10	0.790 ± 0.014	7.198 ± 2.012
20	0.762 ± 0.003	10.689 ± 2.185
50	0.750 ± 0.017	11.640 ± 1.890
100	0.733 ± 0.008	13.652 ± 2.526
200	0.662 ± 0.004	23.862 ± 1.826

The ethanol extract of yellow roots are known to have the greatest inhibition power at concentrations of 200 ppm (23.862%). It means, IC_{50} of yellow root extract is above 200 ppm. The inhibition power of IC_{50} yellow root extract is very weak compared to the inhibition power of IC_{50} glucobay. Glucobay is a synthetic drug that has been commonly used to treat diabetes. The analysis showed that glucobay has a stronger inhibition power, even at concentration of 0.5 ppm, it was able to inhibit the activity of α- glucosidase up to 70.53% with IC_{50} of 0.207 ppm ± 0.033. The inhibition power of glucobay at some variation of the concentrations shown in Table 3.

Table 3. The inhibition power of glucobay towards the activity of α- glucosidase

[glucobay] (ppm)	Absorbance	Inhibition Power (%)
0	0.862 ± 0.018	-
5	0.811 ± 0.016	4.339 ± 0.773
10	0.790 ± 0.014	7.198 ± 2.012
20	0.762 ± 0.003	10.689 ± 2.185
50	0.750 ± 0.017	11.640 ± 1.890
100	0.733 ± 0.008	13.652 ± 2.526
200	0.662 ± 0.004	23.862 ± 1.826

This inadequate inhibition activity of yellow root extract is suspected because of the extract has not been purified or isolated. Some plants have a very strong inhibition activity which shown by the fractions of the active compound. Ying Z *et al*. (2014) [10] reported that the four compounds alkaloids, ie cytidine, 2-(1,2,3,4-tetrahydroxybutyl)-5- (2,3,4trihydroxybutyl) pyrazine, 2- (1,2, 3,4-tetrahydroxybutyl) -6- (2,3,4-trihydroxybutyl) -pyrazine (97), and 2- (1,2,3,4-tetrahydroxybutyl) -5- (1,2,3,4- tetrahydroxybutyl) -pyrazine, isolated from *Morus atropurpurea* leaves has a very strong inhibition with IC_{50} 2.5, 1.9, 2.0, and 7.2 mmol /L, respectively compared with glucobay which has IC_{50} of 9.25 mmol /L..

V. CONCLUSIONS

Yellow root bark extract has a very weak inhibition activity towards α-glucosidase enzyme. The enhancement of inhibition activity of yellow root needs to be done by determining the yellow root extract active compounds which act as inhibitors of the α-glucosidase enzyme.

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