

## Antioxidant Activity and Inhibition of $\alpha$ -Glucosidase from Yellow Root Extract (*Fatoua Pilosa Gaudich*) In Vitro

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### ABSTRAK

Akar kuning (*Fatoua pilosa Gaudich*) merupakan jenis tanaman yang banyak ditemukan di dataran rendah terutama di Jepang, Cina dan biasanya tumbuh di semak-semak kering. Secara tradisional akar kuning (*Fatoua pilosa Gaudich*) digunakan untuk mengobati penyakit diabetes melitus dan sebagai antiinflamasi. Salah satu penyebab penyakit diabetes adalah stres oksidatif. Stres oksidatif dapat dikurangi dengan menggunakan antioksidan. Penelitian ini bertujuan untuk mengeksplorasi aktivitas antioksidan dan penghambatan enzim  $\alpha$ -glukosidase dari ekstrak akar kuning (*Fatoua pilosa Gaudich*). Penentuan kuantitatif fenolik total dengan menggunakan metode folin-ciocalteu dinyatakan sebagai gallic acid equivalents (GAE) per gram ekstrak, kadar flavonoid total dengan metode  $AlCl_3$  dinyatakan sebagai Quercetin equivalen (QE), dan aktivitas antioksidan dengan DPPH (2, 2-difenil-1-pikrilhidrazil) dinyatakan dalam IC<sub>50</sub> (inhibisi konsentrasi). Hasil penelitian menunjukkan bahwa kadar total flavonoid tertinggi terdapat pada ekstrak etanol 96% 199.44 g QE/g ekstrak, sedangkan kadar fenolik total tertinggi pada ekstrak etanol 70% 211.54 mg GAE/g ekstrak. IC<sub>50</sub> tertinggi yang diperoleh dari uji antioksidan ekstrak akar kuning adalah ekstrak etanol 70% 14.62  $\mu$ g/mL dan fraksi paling aktif yang dapat menghambat enzim  $\alpha$ -glukosidase adalah fraksi etil asetat dengan nilai IC<sub>50</sub> 680.54  $\mu$ g/mL.

**Kata kunci:** Akar kuning; Alpha-glucosidase; antioksidan

### ABSTRACT

Yellow root (*Fatoua pilosa Gaudich*) is a type of plant which is found on lowland mostly in Japan, China and usually grow in the dry bushes. Traditionally, the yellow root (*Fatoua pilosa Gaudich*) is used to treat the disease of diabetes mellitus and as antiinflammation. One of the causes of diabetes disease is oxidative stress. Oxidative stress can be reduced by using antioxidants. This research aims to explore antioxidant activity and inhibition of  $\alpha$ -glucosidase enzyme from yellow root extract (*Fatoua pilosa Gaudich*). Quantitative determination of total phenolic by using folin-ciocalteu method declared as gallic acid equivalents (GAE) per gram of extract, levels of total flavonoids with  $AlCl_3$  method declared as Quercetin equivalen (QE), and antioxidant activity in fertilization with the DPPH (2, 2-diphenyl-1- picrylhydrazyl) stated in the IC<sub>50</sub> (concentration inhibition). The Results showed that the highest levels of total flavonoids was in extract ethanol 96% 199.44 g QE/g extract, while the total phenolic levels highest in 70% ethanol extracts 211.54 mg GAE/g extract. The highest IC<sub>50</sub> obtained from antioxidant test of yellow root extrat was 70% ethanol extract 14.62  $\mu$ g/mL and the most active fraction that can inhibit  $\alpha$ -glucosidase enzyme was of ethyl acetate fraction with IC<sub>50</sub> values 680.54  $\mu$ g/mL.

**Keywords:** Yellow root; Alpha-glucosidase; antioxidants

Received: 14-06-2023, Accepted: 25-08-2023, Online: 27-09-2023

### INTRODUCTION

Diabetes mellitus is a metabolic disorder in carbohydrate metabolism characterized by high blood glucose levels in the body (hyperglycemia) due to the inability of the body to produce insulin or cells that do not respond to insulin produced (insulin resistance) (AG, 1994).

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Antioxidants are compounds that can inhibit free radical reactions that cause carcinogenic, cardiovascular and aging diseases in the human body. Antioxidants are needed because the human body does not have excessive amounts of antioxidant reserves, if many radicals are formed, the body will need exogenous antioxidants. Phenolic compounds have various biological effects such as antioxidant activity through reducing agent mechanism, free radical catcher, metal chelating, dampening the formation of singlet oxygen and electron donors. Flavonoids are one of phenolic compounds found in fruits and vegetables. In recent years, it has been proven that flavonoids have great potential against diseases caused by radical catchers.

Antioxidant compounds are inhibitors to inhibit autooxidation. Antioxidant effects of phenolic compounds due to oxidation properties that play a role in neutralizing free radicals (Kadifkova Panovska et al., 2005). Natural antioxidants commonly contained in fruits and plants, one of plants that contain antioxidants is yellow roots. Yellow root (*Fatoua Pilosa* Gaudich) is a type of plant that has 50 cm in height, slightly branched and hairy, oval-shaped leaves with a length 2-6 cm and width 1-4 cm, the ends are widen at the bottom. The fruit is enveloped by enlarged petals, fiber-rooted and yellow. This plant is found in many lowlands, Japan, China and usually grows in dry bushes. (Chiang et al., 2010) stated that the methanol extract of the *fatoua pilosa* Gaudich plant was shown to have antimicrobial activity against *Mycobacterium tuberculosis* H37Rv in vitro. This study aims to determine the total phenolic content, total flavonoids, and antioxidant activity of yellow root extract (*Fatoua pilosa* Gaudich).

## RESEARCH METHODS

### Materials

The materials used in this study were the roots of *fatoua pilosa* Gaudich taken from Waibalun Village, Larantuka District, East Flores Regency, NNT (Nusa Tenggara Timur) Province, Indonesia. Ethanol 96%, ethanol 70%, DPPH (2,2-diphenyl-1-picrylhydrazyl), ascorbic acid, MES (2-(N-morpholino) ethane sulfonic acid) buffer pH 6, methanol 20%, methanol 80 %, Gallic acid, Folin-ciocalteu, Na<sub>2</sub>CO<sub>3</sub> 7.5%, quercetin, AlCl<sub>3</sub> 2%.

### Samples and extract preparations

Yellow root was washed and dried in oven at 50 °C for 24 hours, 60-mesh powder was made using grinder. Yellow root simplicia was extracted by boiling and maceration method. The comparison of yellow root simplicia with solvent was 1:10. Extraction through boiling was performed by boiling the simplicia into the water for 2 hours while maceration was performed by soaking the simplicia into 30% ethanol, 70% ethanol and 96% ethanol. Maceration was carried out for 24 hours with shaker at 150 RPM room temperature. The mixture was separated and filtrate obtained was concentrated using evaporator to gain a water extract, 30% ethanol extract, 70% ethanol extract and 96% ethanol extract.

### Determination Water Content of Yellow Root (*Fatoua pilosa* Gaudich)

The water content of yellow root powder was determined by drying it in the oven. The porcelain cup was dried at 105° C for 5 minutes, then cooled in a desiccator and weighed with analytical balance. 1 gram sample was put into a cup and heated at 105 °C for 3 hours, then cooled in a desiccator for 15 minutes then weighed. Heating was repeated until a constant weight was obtained (Standar Nasional Indonesia, 1992) Water content was calculated by the equation 1.

$$\text{Water content (\%)} = \frac{a-b}{a} \times 100\% \quad (1)$$

By :

a = weight of the sample before heating (g)

b = weight of sample after heating (g)

### **Total phenolic analysis**

The method used was folin-ciocalteu with principle the ability of phenolic compounds to reduce phosphomolibdate in Folin-ciocalteu to form blue molybdenum (Dai and Mumper 2010)

0.1 ml of yellow root extract (in DMSO) at concentration 200 µg / ml was added with 1 ml Na<sub>2</sub>CO<sub>3</sub> 7.5% (in DMSO). The solution was added to 1.25 ml Folin-ciocalteu and homogenized. Imubation was carried out in a water bath at 45 0C for 15 minutes (Javanmardi et al., 2003). The absorbance was measured using a visible beam spectrophotometer at a wavelength of 765 nm (Tripoli).

### **Total flavonoid analysis**

The method used is colorimetric aluminum chloride (AlCl<sub>3</sub>). The principle of this method is based on the reaction between AlCl<sub>3</sub> and the hydroxyl group of flavonoids which will form complex yellow compounds. 0.5 ml of yellow root extract (in methanol) at concentration 500 µg / ml was added into 0.5 ml AlCl<sub>3</sub> 2%. The solution was homogenized and incubated for 10 minutes. The absorbance is measured using a visible light spectrophotometer at 415 nm wavelength (Tripoli).(Vongsak et al., 2013).

### **Determiration of Antioxidant Activity of DPPH Method**

Making DPPH mixture. 1.6 mg of DPPH was dissolved in 10 ml of methanol (0.4 mm) and homogenized with a stirrer for 30 minutes. The homogeneous DPPH solution was added with 10 ml 0.1 M MES buffer pH 7 and 10 ml methanol 20%.

Antioxidant activity test. The stock of yellow root extract (in methanol) was added with 80% methane to make a solution with concentrations 0, 1.25, 2.50, 3.75, 5, 6.25 µg / ml. 1 ml solution was added to 1 ml of DPPH mixture, homogenized and incubated for 30 minutes, then measured the absorbance at 520 nm wavelength. The standard compound used in testing antioxidant activity with the DPPH method was ascorbic acid.(Hasim et al., 2017)

### **Phytochemical Test**

The phytochemical test of water extract, 30% ethanol extract, 70% ethanol extract and 96% ethanol extract included qualitative analysis of alkaloids, flavonoids, saponins, tannins and triterpenoids. (CHUGH et al., 2012; Farnsworth, 1966).

### **Alkaloid Test**

10 mg extract was added into 1 ml 2N HCl and 9 ml aquades, then was heated for 2 minutes and cooled. The filtrate was filtered and accommodated. The filtrate obtained was a test solution for reagents Meyer, Wagner and Dragendorf. Positive result showed with white or yellow sediment in Meyer reagent, brown until black sediment in Bouchardart and brown sediment in Dragendorf reagent.

### **Flavonoid Test**

10 mg sample was reacted with 10 ml water then heated. The mixture was separated and the filtrate was added 0.1 Mg powder, 1 ml concentrated HCl and 1 ml amyl alcohol. A positive test was indicated by the appearance of color in the amyl alcohol layer.

### **Saponin Test**

10 mg sample was put into a test tube, then 10 ml hot water was added then cooled. The test solution was shaken vertically for 10 seconds, then observed for 10 minutes. The

formation of 1-10 cm froth showed the presence of saponins in the sample. At the addition of 1 drop HCl 2N the foam did not disappear.

#### **Triterpenoid and Steroid Tests**

10 mg extract was added with 0.5 ml chloroform, 0.5 ml acetic anhydride, and dripped with concentrated sulfuric acid through the tube wall. The brownish red or purple rings boundary of the two solutions was formed indicated the presence of terpenoids and the green or purple at the solution indicated the presence of steroids or triterpenoids.

#### **Tannin Test**

The test solution was made by reacting 10 mg sample with 50 ml hot water, then heated for 5 minutes and the filtrate was filtered. 5 ml test solution was carried out into a test tube, few drops of FeCl<sub>3</sub> were added. The formation of violet green indicated the presence of tannins.

#### **Phenolic test**

0.5 g sample was dissolved in 5 ml water. Few drops of FeCl<sub>3</sub> were added into solution, dark green or blue solution indicated the presence of phenolic compounds.

#### **Glycoside test**

Carrying out by the Lieberman-Buchard reaction. The extract was dissolved in an ethanol solvent, evaporated over a water bath and then dissolved in 5 ml of anhydride acetic acid then 10 drops of concentrated sulfuric acid were added. The formation of blue or green indicates the presence of glycosides.

#### **Fractionation of Extracts**

Ethanol extract had the highest antioxidant effect. Ethanol extract fractionated by using liquid-liquid extraction with increased polarity of solvents, n-hexane (non polar), dichloro methane, ethyl acetate (semi-polar) and water. 0.5 grams 70% ethanol extract of yellow root was dissolved in 100 mL water, stirred until homogeneous then put into a separating funnel. First, it was fractionated with 100 mL n-hexane. The mixture was shaken every 10 minutes 3 times, then stand for more than 30 minutes to achieve a saturated condition to obtain n-hexane and water layers. n-hexane layer was separated, then the water layer was fractionated with 100 mL dichloro methane, obtained by the dichloro methane and water layer, the dichloro methane layer was separated, then the water layer was fractionated again using 100 mL ethyl acetate, ethyl acetate and water were obtained. After the ethyl acetate layer was separated, the water layer evaporated. fractionation results were concentrated with an evaporator.(Kim et al., 2008).

#### **$\alpha$ -Glucosidase Activity Test**

10  $\mu$ l standard solutions, blanko and sample (concentrations 500, 1000, 1500, 3000, 5000 and 6500  $\mu$ g / mL) were inserted into the microplate reader then was added with 50  $\mu$ L phosphate buffer solution pH 7. Furthermore, 25  $\mu$ l of 15 mM p-nitrophenyl- $\alpha$ -D-glucopyranoside (pNPG) substrate was added three minutes before the test began. The reaction was initiated by adding 25  $\mu$ l  $\alpha$ -glucosidase enzyme with concentration 0.04 U/mL in buffer phosphate (pH 7.0) then incubated at 37 ° C for 30 minutes. The reaction was stopped by adding 100  $\mu$ l Na<sub>2</sub>CO<sub>3</sub> 200 mM. The reaction results were measured by a microplate reader at 410 nm wavelength (Seo et al., 2009; Sugiwati et al., 2009). Acarbose was used as positive control with the same reaction system as the sample. The experiment was carried out in 3 repetitions. % inhibition calculation was performed to determine IC<sub>50</sub> value. The reaction system can be seen in Table 1.

Table 1.  $\alpha$ -glucosidase inhibition reaction system

Reactor	Volume ( $\mu$ l)			
	Blanko	Blanko Control	Sample	Sample Control
Solvent	10	10	-	-
Sample Buffer	-	-	10	10
Substrate Enzyme	50	50	50	50
Buffer	25	25	25	25
	25	-	25	-
	-	25	-	25
	Incubate 37°C 30 minutes			
Na <sub>2</sub> CO <sub>3</sub>	100	100	100	100

### $\alpha$ -Glucosidase inhibition kinetics test (Dixon 1953; Sengupta et al. 2009)

Inhibition kinetics analysis of yellow root extract was carried out using Dixon equation. In this test, two different substrate concentrations were used. Using the plotting technique, the  $K_i$  value will be obtained easily based on linear curve  $1/v$  vs  $[I]$ , besides using this equation to determine the IC50 value. The enzyme concentrations used in this test were the same as the  $\alpha$ -glucosidase inhibitory activity test in the previous stage (500, 1000, 1500, 3000, 5000 and 6500  $\mu$ g / mL). The  $K_i$  value was obtained from the intersection of each linear curve, while the IC50 value for each number of substrate will be obtained from the intersection of each curve with the x axis. The set plate in this test is shown in Table 2.

Table 2. Set of test for inhibition of  $\alpha$ -glucosidase enzyme kinetics

Reactor	Volume ( $\mu$ l)	
	Substrate 1	Substrate 2
Solvent	-	-
Sample	10	10
Buffer	50	50
Substrate	10	15
Enzyme	25	25
Buffer	-	-
	Incubate 37°C 30 minutes	
Na <sub>2</sub> CO <sub>3</sub>	100	100

### Data Analysis

Data were analyzed using Analysis of Variance (ANOVA) and further tested with Duncan 's multiple range test (DMRT) using the SPSS version 16.0 program.

## RESULTS AND DISCUSSION

### Water Content and Extract Rendemen

Water content was measured (Standar Nasional Indonesia, 1992), three times repetition. The water content obtained in this study was 1.43%, indicated that every 100 grams of yellow root contains 1.43 grams of water. The smaller the water content of simplicia means the longer the storage period of simplicia and the better in maintaining the properties of the compounds contained in simplicia.

The extraction process was repeated three times so that the compounds contained in the simplicia can be extracted more optimally. The results of yellow root extract percentage can be seen in Table 2. Based on the results, the water extract has the highest rendement value, it was assumed that most of the phytochemical content in yellow roots was polar.

Table 3. Extraction rendement

Sample	Rendement (%)
Ethanol Extract 30%	13,7
Ethanol Extract 70%	12
Ethanol Extract 96%	11,48
Water	17,9

### Total phenolic analysis

Determination of total phenolic levels using the folin-ciocalteu method, with gallic acid (stable natural phenolics) as standard. Gallic acid reacted with the Folin Ciocalteu reagent to produce yellow, indicated that it contained phenolic then added  $\text{Na}_2\text{CO}_3$  as an alkaline atmosphere giver. During the reaction, the hydroxyl group in the phenolic compound reacted with reagent Folin Ciocalteu, forming a blue molibdenumtungsten complex with structure that is not yet known and can be detected by a spectrophotometer that measured absorbance at a wavelength 765 nm. The more concentrated the color produced, the more phenolic compounds present in the sample. The results of the standard measurement of gallic acid were obtained by linear regression equation  $y = 0.0032x + 0.004$  with a correlation coefficient (r) of 0.994. Determination of phenol content from yellow root ethanol extract was carried out triplo.

Table 4. Total phenolic content

Extract	Total phenolic (mg GAE /g extract)
Ethanol Extract 96%	189,583± 7,046
Ethanol Extract 70%	211,458 ± 9,418
Ethanol Extract 30%	160,416 ± 8,605
Water extract	108,854 ± 10,045

Based on the data in the Table 3, 70% ethanol extract from the yellow root had the highest total phenolic content compared to other extracts, this was due to the polarity of the solution. (Stankovic et al., 2011) stated that the highest phenol content was found in high polarity solvents, 70% ethanol was a high polarity solvent compared to other solvents, so phenol compounds were more soluble in 70% ethanol solvents.

### Total flavonoid analysis

Determination of total flavonoid content using colorimetric aluminum chloride ( $\text{AlCl}_3$ ) method, with quercetin as a standard because quercetin was a flavonoid from the flavonol group which has ketone group of C-4 atoms and also a hydroxyl group on the C-3 and C-5 adjacent. The addition of  $\text{AlCl}_3$  reagent to form a reaction between  $\text{AlCl}_3$  and flavonoid group forms a complex between adjacent hydroxyl groups and ketones.  $\text{AlCl}_3$  will react with ketone groups at C4 and OH at C3, or C5 on flavone or flavonol compounds to form stable yellow complexes. Absorbance was measured at 415 nm wavelength. quercetin standard measurement results obtained linear regression equation  $y = 0.0108x + 0.0803$  with a correlation coefficient (r) 0.992. Determination of the flavonoid content of the yellow root ethanol extract was carried out triplo.

Table 5. Total flavonoid content

Extract	Total flavonoids (mg QE/g extract)
EtOH 96%	199,444 ± 16,667
EtOH 70%	158,703 ± 10,311
EtOH 30%	106,851 ± 12,143
Water extract	51,296 ± 11,264

Based on the data in the Table 3, extract 96% ethanol of yellow roots had the highest total flavonoid content compared to other extracts. This was due to the solubility of flavonoids which were more soluble in solvents with moderate polarity, 96% ethanol had a medium polarity solvent, so flavonoid compounds were more soluble in 96% ethanol solvents.

#### Determination of Antioxidant Activities With DPPH Method

The DPPH method uses 2,2-diphenyl-1-picrylhydrazyl as a source of free radicals. The principle was the reaction of hydrogen captured by DPPH from antioxidants. The antioxidant activity in DPPH was expressed with IC<sub>50</sub> (inhibitory concentration). IC<sub>50</sub> is a number that shows the concentration of extract which can inhibit the DPPH activity by up to 50%. The smaller IC<sub>50</sub> values indicate higher antioxidant activity (Blois 1958 referenced in (Hanani et al., 2005)). The results showed that the antioxidant activity of the yellow root extract was significantly different in the alpha test = 0.05. IC<sub>50</sub> values of water extract, ethanol extracts 30%, ethanol extracts 70%, and ethanol extract 96% were 15.27, 17.31, 14.62, 15.35 µg / mL (Figure 1), which showed very strong antioxidant activity.

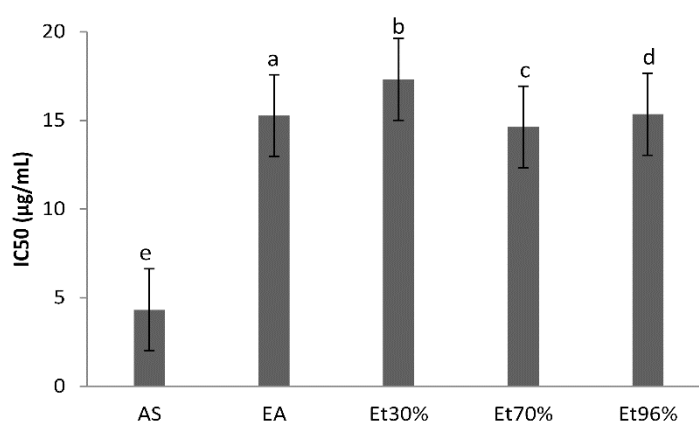


Figure 1. Yellow root antioxidant activity expressed with IC<sub>50</sub> value. Different letters show antioxidant activity significantly different from Duncan's test at alpha = 0.05

The smaller IC<sub>50</sub> value, the higher antioxidant activity. Very strong antioxidant compound has IC<sub>50</sub> value less than 50, strong antioxidant (50-100), moderate antioxidant (100-150) and weak antioxidant (151-200) (Badarinath et al., 2010). Figure 1 shows the IC<sub>50</sub> value of each extract was less than 50 which means the antioxidant activity of the yellow root was very strong. The ability of the solvent to extract the secondary metabolites present in the sample underlies the different activities of each extract (Teh et al., 2014)

#### Phytochemicals from Yellow Roots

The phytochemicals analysis were alkaloids, flavonoids, saponins, tannins, phenolics, glycosides, steroids and triterpenoids test. Positive results can be seen from the formation of deposits or changes in color during testing. The yellow root ethanol extract positively contained alkaloids, flavonoids, tannins, saponins, phenolics, triterpenoids and steroids. The phytochemical test results of yellow root ethanol extract can be seen in Table 4.

Table 6. Phytochemical test results of yellow root ethanol extract

Chemical Content	Test results
Alkaloids	++
Flavonide	+++
Tanin	++
Saponin	+
Phenolic	+++
Triterpenoid	++
Steroids	++
Glycosides	-

Description : (+) = a little; (++) = enough; (+++) = many; (-) = does not contain compounds

### Fractionation

Fractionation results with several solvents can be seen in Table 7.

Table 7. Results of yellow root fraction

Sample	Rendemen (%)
N-hexane fraction	10,4
Ethyl acetate fraction	27
Dichloro methane fraction	18,2
Water fraction	38,4

Based on the yield of extracts, the water extract has the highest rendement value, it is assumed that most of the phytochemical content in yellow roots is polar.

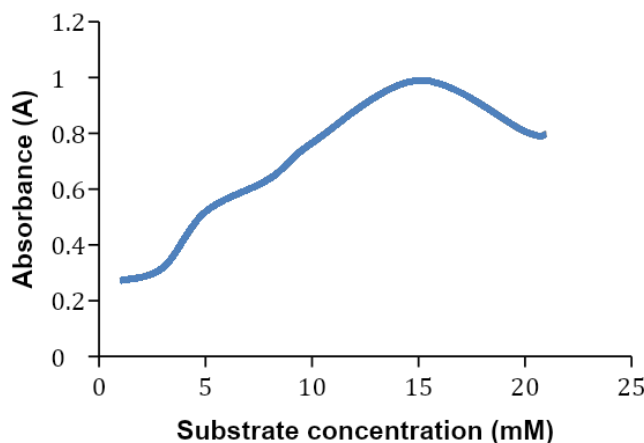


Figure 3. Graph of absorbance optimization with variations in substrate concentration

The optimization test for substrate concentration showed 15 mM substrate concentration as the optimum concentration. The antidiabetic activity in this study was carried out through the inhibition of  $\alpha$ -glucosidase enzyme, an enzyme that catalyzes the final reaction of carbohydrate absorption in the intestine. The inhibition test of  $\alpha$ -glucosidase activity used an enzyme solution with concentration 0.04 U/mL, a substrate solution with concentration 15 mM. The test was conducted to determine the inhibitory power of 70% ethanol extract and fraction of the yellow root extract to  $\alpha$ -glucosidase by looking at IC50



values. IC<sub>50</sub> is the concentration needed to inhibit 50% of enzyme activity. Acarbose was used as a comparison. The principle of this test is the hydrolysis reaction on the substrate p-nitrophenyl- $\alpha$ -D-glucopyranoside producing  $\alpha$ -D-glucose and p-nitrophenol which produced yellow color. The yellow color produced by p-nitrophenol is an indicator of  $\alpha$ -glucosidase inhibitory activity, if the yellow color fades, it shows high inhibitory activity (Sugiwati et al., 2009). The results showed that the test carried out on acarbose obtained an IC<sub>50</sub> value 1.213  $\mu$ g / mL. Alpha-glucosidase activity from acarbose, 70% ethanol extract yellow root and fraction is shown in Figure 2.

### Inhibition of $\alpha$ -glucosidase enzyme activity

The optimization test for substrate concentration was carried out using variations in substrate concentration 1, 3, 5, 8, 10, 15, 20 and 25 mM. The reaction speed will increase with increasing substrate concentration, until the optimizing point was obtained. At this boundary called the maximum speed ( $v_{max}$ ), when the enzyme becomes saturated by its substrate, and it cannot function faster (Lehninger 1990). The test results showed that the optimal substrate concentration was 15 mM, because at a concentration of 20 mM the saturated substrate binds to the enzyme. The graph of the results of the automation of substrate concentration was shown in Figure 4.

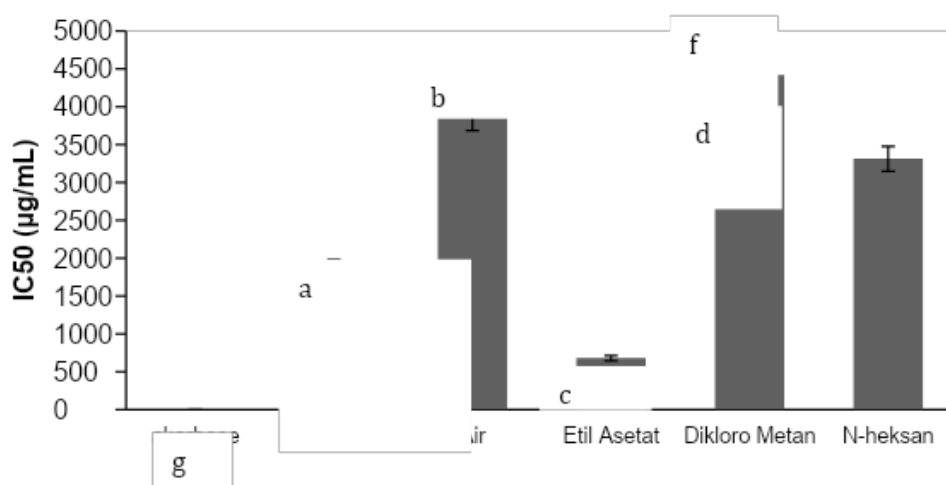


Figure 4.  $\alpha$ -glucosidase inhibition of 70% ethanol extract and yellow root fraction expressed with IC<sub>50</sub> values. Different letters show that  $\alpha$ -glucosidase inhibitory activity is significantly different from Duncan's test at  $\alpha = 0.05$ .

Figure 4 shows that the fraction with the highest  $\alpha$ -glucosidase inhibition was ethyl acetate fraction with IC<sub>50</sub> values 680.54  $\mu$ g / mL, this showed that compounds that have the ability to inhibit  $\alpha$ -glucosidase were more semi-polar. The difference in sample inhibition activity due to the differences in the content of secondary metabolites obtained from the results of fractionation with different solvents. The ability of extracts to inhibit carbohydrate hydrolysis enzymes due to the presence of phenolic compounds that can bind to enzyme proteins (Zhang et al., 2015).

### $\alpha$ -Glucosidase inhibition kinetics

Ethyl acetate fraction had a higher  $\alpha$ -glucosidase inhibitory activity used as an inhibitor to determine the type of enzyme inhibition. The inhibition kinetics test was carried out using 10 and 15 mM substrates. The determination of the inhibition kinetics of the  $\alpha$ -glucosidase enzyme aims to determine the type of inhibition of the sample against the enzyme. The mechanism of enzyme inhibition was determined by Dixon method, this method used a variation of samples and substrates (Yoshino & Murakami, 2009). The

product concentration value was obtained using linear equations on the p-nitrophenyl standard curve. The test results using the Dixon method, obtained a plot in Figure 3 which shows the intersection between two lines on the x-axis, which indicates that the sample has a non-competitive inhibitory mechanism.

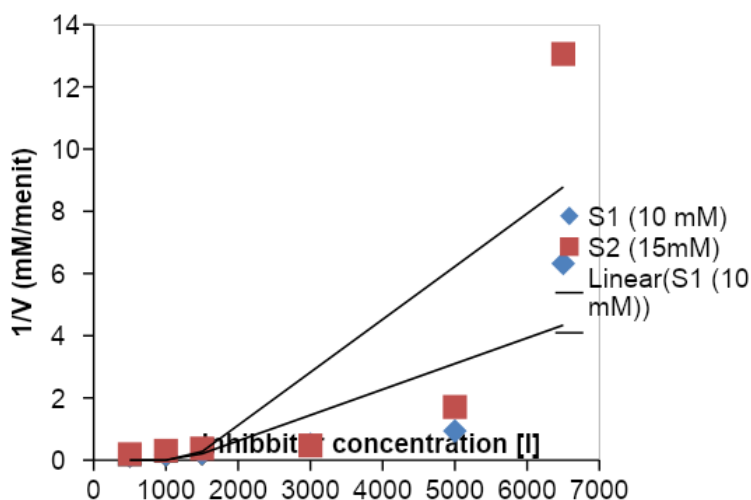


Figure 5. Dixon plots of ethyl acetate fraction

In non-competitive inhibition, inhibitors work by attaching themselves outside the active side of the enzyme, which causes the shape of the enzyme to change and the active side of the enzyme cannot function, this causes the substrate can not bind to the active side of the enzyme. The mechanism of non-competitive inhibition can be seen in Figure 4. The value of the inhibition constant ( $K_i$ ) can be determined by the intersection between the two lines in the Dixon plot. The  $K_i$  value shows how easily the enzymes break down from the complex [ES] (Bisswanger, 2008).

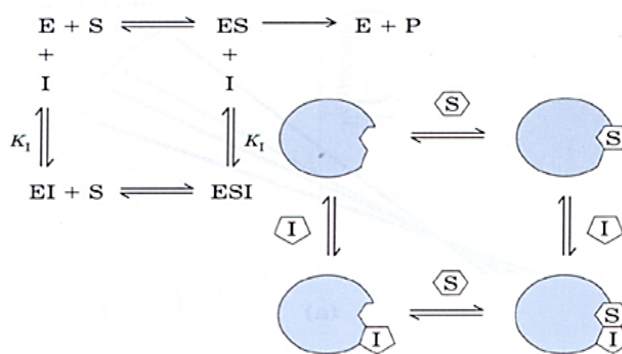


Figure 6. Mechanism of non-competitive inhibition

### CONCLUSION

The results showed that the highest total flavonoid content in 96% ethanol extract was 199.44 g QE / g extract, while the highest total phenolic content in 70% ethanol extract was 211.54 mg GAE / g extract. The highest IC<sub>50</sub> value was obtained from antioxidant testing of yellow root extract on 70% ethanol extract with IC<sub>50</sub> value 14.62 μg / mL. The results of the α-glucosidase inhibition test showed that ethyl acetate fraction was the most active fraction with IC<sub>50</sub> value of 680.54 μg / mL. The sample of yellow root ethyl acetate fraction has an uncompetitive inhibition mechanism. It is necessary to analyze the components of the compounds contained in 70% ethanol extract yellow root and ethyl acetate fraction. It needs to test antidiabetic activity in vivo.

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