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Antidiabetic Activity Test of the Ethyl Acetate Fraction of Arrowroot Root Extract (Marantaarundinaceae) Enzyme Inhibition A-Glucosidase

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ABSTRACT

According to the data World Helath Organization (WHO) there are approximately Around 220 million people in this world suffer diabetes. Although this disease is not contagious, diabetes mellitus can be emergency. One of the food plants that has water-soluble polysaccharides (PLA) and a low glycemic index value compared to other tubers is arrowroot tuber.(Maranta arudinaceae L) contains high enough nutrients as processed food ingredients, with a flour content of 19.14-21.7%, 25-30% carbohydrate content, a low glycemic index is below the value of rice, wheat, potatoes and cassava which is 96, 100 , 90 and 54 and arrowroot tubers of 32 , can increase insulin sensitivity to secondary metabolites and slow down glucose absorption and prevent insulin hormone secretion from the pancreas, so that blood sugar does not increase within 2 hours after eating. This study aims to determine the magnitude of the enzyme inhibitionα-glucosidaseand value (IC50) from the ethyl acetate fraction of arrowroot bulbs with a positive control, namely acarbose

The method was carried out by extraction for 4x 24 hours as much as 420 grams of sample with 2 Liters100ml 96% ethanol which was then fractionated using ethyl acetate. With test results non-specific parmeter ash content was 6.96%, drying shrinkage was 7.46%, moisture content was 5.91%, ethanol soluble essence content was 6.24%, water content test was 9.16%. Results of analysis of the activity of antidiabetic enzymesα-glucosidasein the ethyl acetate fraction of arrowroot tubers, the IC_{50} value was obtained50 of 12255,4 ppm, and a standard comparison of acarbose with a value of IC_{50} 0.2008v,From data analysis of enzyme inhibition activityα-glucosidaseArrowroot tubers have less potential to inhibit enzymesα-glucosidasebut still has the potential as an anti-diabetic because it has the bioactive PLA (water-soluble polysaccharide) compound, its metabolite compounds and its low glycemic index value compared to other flours.

KEYWORDS: α-Glucosidase , arrowroot tuber, ethyl acetate fraction, antidiabetic

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I. INTRODUCTION

Diabetes is one of the biggest diseases that can threaten human health since the 21st century. From data *World Helath Organization (WHO)* there are approximately Around 220 million people in this world suffer diabetes disease. Although disease this no caninfectious, diabetes even Cancanthreaten life According to *International Diabetes Federation* (IDF), number of cases death in diabetes Of Indonesia year 2015 reachas much as 184.985 soul. Matter This show that diabetes is problem health biggestand need handling Which optimal with quick (Ariani et *a*l.,.2017).

In general, diabetes can be managed in three ways, including following a diet. Controlling diabetes through diet is the same as implementing a healthy and balanced diet (nutritional therapy) in a controlled manner by consuming foods that are low in sugar and contain water-soluble polysaccharides (PLA), which can reduce the efficiency of absorption of carbohydrates, thereby allowing the insulin response to be stable. (Yuniastuti et al., 2018).

One of the lists of plants and food ingredients that contain water-soluble polysaccharides (PLA) and a low glycemic index value compared to other tubers is arrowroot tubers.*(Maranta arudinaceae L)*, which has a high nutritional value of processed food, such as starch of 19.14-21.7%, protein of 1.0- 2.2%, water of 69.072.0%, fiber of 0.6 -1.3%, ashcontent of 1.3-1.4% per sugar, soluble dietary fiber of 9.79-13.7 (Yuniastuti*et al*., 2018). And contains metabolite components such as saponins, phenols and flavonoids (Tiara Fajar Budiarti, 2019).

Not only do they have good nutritional value, but arrowroot tubers have many benefits, such as the juice from arrowroot tubers can be used as an antidote for bee stings and snake venom and can treat diarrhea. Not only that, arrowroot tubers can be processed into various foods, and are widely used in the food industry. babies and special food for sick people, making wet and dry cakes, arrowroot jenang, arrowroot chips, arrowroot chips which can be digested by diabetics and can reduce stomach problems and intestinal problems (Nurhayati et al. *al*., 2022).

From the research results of Yuniastuti et*al*. (2017b) can report that arrowroot tubers have a low glycemic index of 15, so they can reduce sugar blood also plays a role in convertcarbohydrate become glucose. With inhibited activity on enzyme on *αglucosidase*,solution carbohydrate complex disaccharide as well as monosaccharides becomedelayed, possible much sugar blood canreturn become normal

The glycemic index (GI) is a value to measure the degree to which food can increase blood glucose levels after eating. In general, foods with a high GI can increase blood sugar levels greatly, while foods with a low GI can increase blood sugar levels gradually.*(*Septianingrum *et al*., 2016).

with a high IG value of more than 70, a medium IG value of 55 to 69, and a low IG value of less than 55 (Diyah*et al*., 2018). The presence of water-soluble polysaccharides and dietary fiber are two bioactive ingredients which provide the ability to lower blood glucose levels (Saputro & Estiasih, 2015)

II. TOOLS AND MATERIALS

This research was carried out at the Research Laboratory of the Faculty of Pharmacy, University of 17 August 1945 Jakarta which is located on Sunter Permai Raya, North Jakarta City, Indonesia Testing *α-glucosidase enzyme* The in vitro method was carried out at the Biopharmaceutical Laboratory which is located on Taman Kencana Bogor Tengah, West Java City.

As well as plant determination on arrowroot tubers, it is carried out at the UPT Batu Herbal Materia Medica Laboratory which is located on Jalan Lohor No. 87, Pesanggrahan, District. Batu City, East Java.

The tools used include : *rotary evaporator*, Blender, *microplate reader,* analytical balances, Ovens, Furnaces,*Moisture Balance*. Spectrophotometers, as well as commonly used laboratory equipment . The materials used include:Enzymes*α-glucosidase*comes from recombinant s*p-Nitrophenyl-α-D-glucopyranosidases*(PNPG*),* The comparison is Acarbose, phosphate buffer solution with pH 7,*Bovine Serum Albumin*(BSA) , Ethanol 96%, Na2CO3, Ethyl Acetate, N-Hexane, NButanol, Aqua Dest, HCl, Mg, FeCl3 , HCL2 N*, Libermann- Burchard*.

III. THE METHODS ENZYM *A -GLUKOSIDASE*

a. Sample testing ethyl acetate fraction of arrowroot tuber extract (*Maranta Arudinaceae*)

50 mg of the extract sample was diluted at a concentration of 50.000 ppm , 25,000 ppm ,12,500 ppm , 6,250 ppm , 3,125 ppm,1,562.5 ppm, 781.25 ppm and then added with 50°L phosphate buffer ph 7 And also with 25 µl p-Nitrophenyl-3-D-glucopyranosidasi (PNPG) with a concentration of 20mm, incubated for 30 minutes at a temperature of 37 ° C after that add 25 µl enzyme solution with a concentration of 0.01 U/Ml. After the reaction has been stopped add 100 µl of 0.1 M solution of Na2CO3 at a concentration of 200 mM. The sample solution can be read on a microplate reader with a wavelength of 410 nm.

b. Sample testing of Blanco

50 µl phosphate buffer (Ph 7), 10 µl dimethyl sulfoxide (DMSO) were added, 25 µl p-nitrophenyl- 3 -Dglucopyranoside (PNPG) at 20 mM and 25 µl enzyme solution with a concentration of 0.01 U/Ml.The mixture was then incubated at 37°C for 30 minutes. At the end of the incubation, add 100 µl of a solution of Na2CO3 with a concentration of 200 mM.The sample solution can be read with a microplate reader at a wavelength of 410 nm.

c. Sample testing of Acarbose

Acarbose solution with concentration. 10 ppm, 5 ppm, 1 ppm, 0.5 ppm, 0.1 ppm, and then added 50 µl phosphate buffer (Ph 7), as well as 25 µl p-nitrophenyl -3-D-glucopyranosidate (PNPG) and 25 µl enzyme solution with a concentration of 0.01 U/ml. Then incubated at a temperature of 37°C for 30 minutes. After incubation, 100 µl of Na2CO3 0.1 M solution with a concentration of 200 mM is added. The sample solution can be read using a microplate reader at a wavelength of 410 nm.

IV. *RESULTS*

A. Test result Extract Characteristics

In this research, the specific parameter tests were determined by organolptic examination using the five senses, including: smelling the new extract, tasting the taste of the extract, and seeing the color and shape of the extract that had been obtained from 96% ethanol extraction and the ethyl

acetate fraction in arrowroot tubers which can be seen in table 1. following as follows:

Table 1. Organoleptic Results of 96% Ethanol Extract & Ethyl Acetate Fraksi Garut tubers (*Maranta arudinaceae*)

No.	Parameter	Results
1	Form	Thick
2	Color	Chocolate
3	Smell	Special
4	Feel	Bitter

B. Non-Specific Parameter Test Results his study the determination of non-specific parameters of arrowroot tubers include determination of ash content, determination of dry shrinkage, determination of water content, determination of dissolved ethanol content and determination of water soluble extract content.the results obtained can be seen in Table 2 as follows :

Table 2. Results of Non SpecificParameters of Garut tubers (*Maranta arudinaceae*)

No.	Parameter	Results
1	Ash content	6,96 %
2	Dry shrinkage	7,46 %
3	Water contet	5,91 %
4	dissolved ethanol content	6,24 %
5	Water Soluble Eextract content	9,16 %

The extraction of arrowroot tubers using ethanol 96% solvent, with the resulting extract weight of 22.95 grams and yield value obtained 5.46% for fractionated extract using ethyl acetate solvent extract weight of 300 mg with a yield value of 3.33%

C. Phytochemical Screening Test Results

The phytochemical screening carried out in this study aims to determine the presence of compounds The metabolites contained in arrowroot tubers, the metabolite compounds tested include: flavonoids, tannins, terpenoids/steroids and saponins. and the results obtained can be seen in table 3 as follows.

D. Acarbose Comparator Test Results

Before the enzyme inhibition test is carried out*α-glucosidase* of the extract, it is necessary to carry out a comparative test with acarbose to compare the IC_{50} value$_{50}$ acarbose with IC_{50} value on samples of the ethyl acetate fraction of arrowroot tuber extract. Acarbose was chosen as a comparison because it has been an internationally recognized benchmark for a long time.

When testing enzyme inhibitory activity *α- glucosidase*, usually acarbose is used as a positive control, because acarbose is a complex oligosaccharide compound that is a potential competitive inhibitor of enzymes. *α-glucosidase* who works in *brush* to break down starch, dextrin, maltose, and sucrose to produce digestible monosaccharides. And inhibits pancreatic alpha-amylase which reduces elevated postprandial glucose levels. Acarbose, at a dose of 150–160 mg per day, is an oral antidiabetic for people with type 2 diabetes mellitus, with side effects reported in sufferers experiencing flatulence, diarrhea and digestive problems (Andi Early Febrinda *et al*., 2013).

Based on the optimization results and linear regression equation of acarbose, namely y=11.839x+69.006 and the correlation coefficient value obtained is 0.995, the IC50 value obtained from the linear regression equation, namely 0.2008 ppm. Limier regression equation curve of Inhibitory Activity *α-glucosidase* A comparison of acarbose can be seen in Figure 1

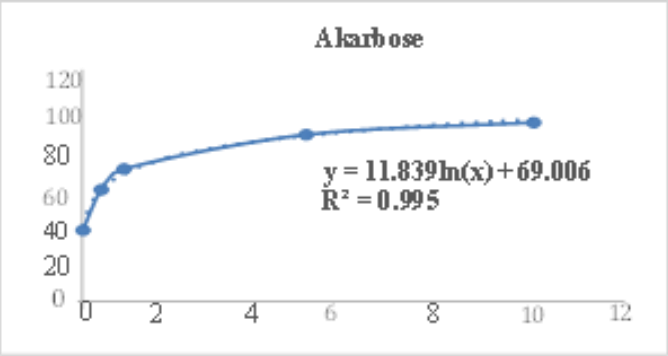

Figure 1. Inhibitory Activity Curve *α-glucosidase* by Acarbose

E. Test result Ethyl Acetate Fraction of Garut Tuber Extract (Maranta arundinacea)

Measurement of enzyme inhibitory activity *α-glucosidase* carried out at a wavelength of 410 nm because the maximum absorption of p-nitrophenol is at a wavelength of 400-410 nm. In this research on arrowroot tubers, tests were carried out with various concentrations, namely 50,000 ppm, 25,000 ppm, 12,500 ppm, 6,250 ppm, 3,250 ppm, 1,562, 5 ppm, to the lowest concentration of 781.25 ppm. The aim is to see the effect of extract concentration on enzyme inhibition. The greater the resistance, the lower the IC_{50} value$_{50}$ which is obtained. This shows that the inhibitory effect is greater and vice versa. (Meila & Noraini, 2017). Based on the optimization results and linear regression equation data, the result of the ethyl acetate fraction of arrowroot tubers is

y=21.014x–147.82, so the correlation coefficient is 0.9949. Based on the linear regression equation, the IC_{50} value is obtained$_{50}$ amounting to 12255.4 ppm. Limier regression equation curve of Inhibitory Activity *α-glucosidase* The Ethyl Acetate Fraction of Garut Tuber Extract can be seen in Figure 2.

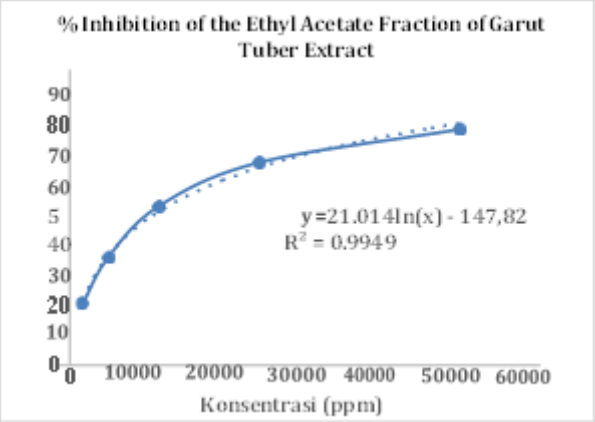

Figure 2. Inhibitory Activity Curve *α-glucosidase* Garut Tuber Ethyl Asetat Fraction

V. DISCUSSION

It can be seen in figure 1 and figure 2 and based on the IC value50 which is produced using*microplate reader* showed that acarbose had better enzyme activity than the ethyl acetate fraction in arrowroot tuber extract. Ability to inhibit enzymes*α- glucosidase* can be different for each plant. These differences can be caused by several factors, including differences in the type of solvent used, the concentration used, and the secondary metabolites contained. Although in this study the ethyl acetate fraction showed enzyme activity *α-glucosidase* high, but the inhibitory power is still lower than acarbose. This is because the ethyl acetate content of arrowroot tubers is the result of fractionation of extracts which are mixed compounds. This allows the ethyl acetate fraction to be not optimal, resulting in an imbalance between other chemical metabolite compounds in arrowroot tubers. (Mustika *et al*., 2021).

How ever, it is known that the arrowroot plant still has potential for diabetes sufferers because the bioactive compounds in arrowroot flour contain starch which is classified as a polysaccharide, namely PLA (water soluble polysaccharide), which can lower blood sugar by improving the digestive tract and slowing down the decline in blood sugar levels and prevents glucose absorption so that it can control the breakdown of glycogen resulting in a decrease in glucose levels and an increase in insulin levels (Yuniastuti*et al*., 2018).

The low glycemic index of arrowroot tubers is below the Ig value of rice, wheat, potatoes and cassava, namely 96, 100, 90 and 54, carbohydrate content is 25-30%, starch as a raw material substitute for wheat, can increase insulin sensitivity to secondary metabolites and slows glucose absorption and prevents the secretion of the hormone insulin from the pancreas, so that blood sugar does not increase within 2 hours after eating. Decreased insulin sensitivity can be caused by decreased insulin sensitivity, decreased insulin requirements and decreased glucotoxic effects due to pancreatic β cell activity, cell dysfunction (Yuniastuti*et al., 2018*).

By inhibiting enzyme activity*α-glucosidase*, can slow glucose absorption after meals, thereby preventing postprandial hyperglycemia. Enzyme *αglucosidase* including maltate, isomaltase, sucrose, lactase and*α-dextrinase*, will break down carbohydrates and convert them into simpler sugars, which are then absorbed by the body and increase blood sugar levels, which can reduce the absorption of monosaccharides and increase blood sugar after eating. so that it can increase the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) to regenerate damaged pancreatic β cells and overcome insulin deficiency (Eryuda & Soleha, 2016). Chemical content or metabolite compounds such as alkaloids, flavonoids , tannins , saponins , Quinones , and steroids/ terpenoids are responsible and become a significant factor in the inhibition of the enzyme α-glucosidase (appendix 21) (Yuniarto & Selifiana, 2018).

In addition to low glycemic index, arrowroot tubers also have bioactive compounds that are good for patientsdiabetes mellitus. Arrowroot flour contains 2.16 mg / 100g diosgenin, 3.98% water soluble polysaccharide,1.49% water insoluble dietary fiber, and 1.12% water soluble fiber (Yogananda and Estiasih, 2016). One of the hydrocolloid compounds that can increase the viscosity of digestion are watersoluble polysaccharides. (Estiasih, etal., 2012).

Dietary fiber has the property to lower postprandial blood glucose levels and insulin evel. Dietary fiber and water soluble polysaccharides can reduce glucose absorption in digestion, resulting in emphasis on blood sugar. Diosgenin is an apogen steroids, a bioactive compound from the triterpenoid group.

Diosgenin has a hypoglycemic effect by reducing the action of maltase, transaminase and lactase enzymes.This compound can reduce the work of disaccharides in the intestine and inhibit the breakdown of carbohydrates into monosaccharides (Patel et al., 2012). According to Ghosh et al., (2014) diosgenin has potential to treat diabetes diabetes because it can inhibit glucosidase and amylase. The work of these two enzymes that break down starch into simple sugars. (Nur Mahwita Adi Setyaningrum, 2022)

CONCLUSIONS

Based on the results of the data analysis that has been obtained, it can be done concluded that the results of the antidiabetic activity test of the ethyl acetate fraction of arrowroot extract (Maranta arudinaceae) with IC_{50} values amounting to 12255.4 ppm is not effective in inhibiting the enzyme*α-glucosidase*However, arrowroot tubers still have

great potential in diabetes therapy because of their low PLA (water-soluble polysaccharide) and Ig content when compared to other flours.

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