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Article

Phytochemical Screening and In-Vitro α-Glucosidase Inhibitory Activity Analysis of Ethanol Extract of *Mangifera quadrifida*

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Abstract: Introduction: Type 2 Diabetes Mellitus (DM) is a metabolic disorder characterized by hyperglycemia caused by B cell dysfunction, insulin resistance, or both. Indonesia and worldwide have an increasing incidence that leads to a significant socio-economic burden. The effective treatment of Type 2 DM with minimal side effects is still challenging. This research aims to identify phytochemical properties in ethanol extract of *Mangifera quadrifida* and the effectivity as an alfa-glucosidase inhibitor beneficial to research on the treatment of type 2 DM. Method: *Mangifera quadrifida* was extracted by ethanol as solvent. The phytochemical analysis is conducted by phytochemical screening methods and thin-layer chromatography (TLC). The antidiabetic properties in ethanol extract of *Mangifera quadrifida* is tested by inhibitory activity on alpha-glukosidase. Result: The ethanol extract of *Mangifera quadrifida* contains tanin, triterpenoid, flavonoid, and glycoside. TLC test on the ethanol extract of *Mangifera quadrifida* was detected 4 points (Rf 0,15; 0,464, 0,511; and 0,63). The IC50 to α-glucosidase of *Mangifera quadrifida* and the acarbose as positive control are 18,19 ppm and 4,88 ppm respectively. Discussion: Tanin, triterpenoid, flavonoid, and glycoside of *Mangifera quadrifida* have antidiabetic properties. The result on IC50 value in ethanol extract of *Mangifera quadrifida* is among the active class of antidiabetic group although the value is higher the acarbose. Conclusion: Ethanol extract of the *Mangifera quadrifida* has phytochemical compunds that have antiadiabetic potential. Acarbose has better IC50 to α-glucosidase compared to Ethanol extract of *Mangifera quadrifida*.

Keywords: *Mangifera quadrifida*, α-glukosidase, type 2 diabetes mellitus, antidiabetic properties.

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1. Introduction

Diabetes is a group of metabolic diseases which characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both.1 In 2014, there were 422 millions people or around 8.5% from population of adult that live with diabetes globally. South East Asian and Western Pacific Regions had the largest numbers among of the half diabetes cases in the world.[2] Indonesian prevalence of diabetes mellitus in 2018 was 20.4 millions people or equal with 8.5% population in Indonesia.[3]

Mechanism of diabetes mellitus is related to chronic hyperglycemia that can cause variety of long term damage, dysfunction, and failure of some organs. Organs which frequently affected is eyes, kidneys, nerves, heart, and blood vessels.[1] Complication caused by diabetes mellitus will resist productivity and also decrease the quality of life. The amount of person with diabetes also can impact the national economic and health.[4]

Governance principle with minimal side effects still become challenge. Nowadays, governance of diabetes mellitus is done by healthy lifestyle (physical activity or medical

nutrition therapeutics) along with pharmacology agent consumption, like anti-hyperglycemic oral or injected drugs. Diabetes mellitus treatment in present, use pharmacology agent, which covers almost 86% from all diabetes mellitus treatment in Indonesia.[5] There are lack of using pharmacology agent in Indonesia, such as undetermined the level of obedience and long term effect from consumption of pharmacology agent available at the moment.[6,7]

The cause of blood glucose level increase after eat is started with hydrolysis of long chain complex carbohydrate into simple chain glucose molecules, then absorbed to intestinal cells through blood glucose increasing process. Strategy to solve blood glucose increasing can be done with decrease the production and absorption of monosaccharide using the inhibition of carbohydrate digesting enzymes. α-amylase and α-glucosidase inhibitor have shown the significantly decrease of postprandial increase from blood glucose.[8]

There are some studies that explain anti-diabetes activity in plants, one of them are α-amylase or α-glucosidase inhibition activity. Plant which show α-amylase or α-glucosidase inhibition activity is Mangifera indica.[9] Asam kumbang (*Mangifera quadrifida*) has same genus with Mangifera indica, and wide-spread in Indonesia (Kalimantan, Java, Sumatra), Malaysia, Brunei, and Singapore. This species is found often on lowland also backyard and consumed by villagers.[10] The ability of *Mangifera quadrifida* inhibition for α-amylase or α-glucosidase still unknown. This study is aimed to identify phytochemical and examine α-amylase and α-glucosidase activity in ethanol extract from *Mangifera quadrifida* fruit..

2. Results

2.1. Phytochemical test

Phytochemical test is a test to detect organic compounds contain in the extract. The phytochemical test results of the ethanol extract shown at Table 1. Ethanol extract of *Mangifera quadrifida* are contain flavonoid, triterpenoid, tannin, saponin, and glycoside.

The first chemical compounds contained in Mangifera extract are flavonoids. Research by Anila et al (2020) showed the presence of flavonoids in the ethanol extract of Mangifera indica. Favonoids belong to a class of polyphenolic chemical compounds that have a carbon skeleton structure of C6-C3-C6 equipped with a pyran or chroman ring carrying a second benzene ring.[11,12] In its structure, flavonoids have a sugar group that can make it easy to dissolve in polar and semipolar compounds. This reason supports the presence of flavonoids in ethanol extracts.[13]

Triterpenoids are part of the largest group of active plant products, which are chemically classified based on the number of isoprene subunits [C5H8]n where n is the number of isoprene units. Triterpenoids have been shown to act in opposition to diabetic vascular dysfunction, retinopathy, and nephropathy.[14] Saponins in several studies have shown antidiabetic activities including increasing insulin secretion and action, regenerating cells, increasing hepatic glycogen storage, and as efficient antioxidants.[15]

Glycosides in the ethanol extract of Mangifera were also discovered by Wilberforce et al. Glycosides are polar compounds because they have at least one sugar moiety in their molecular structure. Based on the principle like dissolves like, most glycosides can be isolated with polar solvents, such as methanol, ethanol, acetone, and water. Therefore, glycosides can be identified in the ethanol extract of *Mangifera quadrifida*.[16,17]

Tannins are bioactive compounds of plant secondary metabolites that have various benefits as antidiabetic compounds. Tannins could be used as an inhibitory agent of -amylase and -glucosidase and have potential to be used in type 2 DM patients who need certain inhibition of post-prandial glucose elevation.[18]

Table 1. Phytochemical test result of ethanol extract of *Mangifera quadrifida*

No.	Metabolite	Observations	Interpret.
1	Alkaloid	No orange precipitate is formed	-
2	Flavonoid	Color change into green	+
3	Triterpenoid	Formation of a brownish ring and violet color	+
4	Steroid	No color change	-
5	Tannin	Color change into blackish green	+
6	Saponin	Stable foam was formed after shaking for 10 minutes and adding 1 drop of HCl2N	+
7	Glycoside	Color change into green	+

2.2.Thin Layer Chromatography Analysis

Thin Layer Chromatography (TLC) analysis of ethanol extract of *Mangifera quadrifida* carried out using mobile phase eluent n-hexane : ethyl acetate with ratio 5:1. The TLC analysis showed that were 4 spots in the ethanol extract (Tabel 2). The value of Rf in Table 2 also shows how far the extract has moved from starting point.

Chromatographic technique has been used in many studies to isolate and purify the compounds responsible for the biological activity of an extract. Thin layer chromatography (TLC) used in this research with ethanol:ethyl aceta as the mobile phase in a ratio of 10:1, while the stationary phase used is silica. The TLC test results that have been read under UV light have shown the presence of 4 spots with an Rf of 0.15; 0.464; 0.511; and 0.63.

In TLC, the thin layer plate acts as the stationary phase and the solvent liquid as the mobile phase moves by capillary action along the stationary phase. The distribution of chemical compound groups follows the suitability of the chemical compound structure and polarity in the stationary phase and the mobile phase. The secondary metabolites that have been separated form a point in the stationary phase and are assessed for retention factor (Rf).

The law of solubility like dissolves like based on polarity underlies the distribution of secondary metabolites. Secondary metabolites with smaller Rf values have more polar properties because they are retained longer in the stationary phase. On the other hand, secondary metabolites with higher Rf values tend to be more non-polar because their polarity with non-polar solvents makes them move faster. The difference in the Rf value of the secondary metabolites can be observed in the ethyl acetate extract (Table 2.). In ethanol extract there are secondary metabolites with the smallest Rf value, namely 0.15, and the largest Rf value is 0.63. This shows that the ethanol extract contains secondary metabolites that are more polar and more non-polar.

Table 2. Thin Layer Chromatography Results

Spot	Rf
1	0.154
2	0.464
3	0.511
4	0.630

2.3. In vitro α-Glucosidase Inhibition assay of Mangifera quadrifida ethanol extract and acarbose

The inhibition assay was conducted on six concentrations of the ethanol extract and acarbose. Acarbose was used as positive control. The inhibition activity of acarbose and ethanol extract shown at Table 3 respectively.

Table 2. Thin Layer Chromatography Results

Concentration (ppm)	Sample Inhibition Activity (%)	
	Akarbosa	Ethanol extract
100	128,85 ± 5,45	131,07 ± 2,61
50	99,48 ± 2,98	101,17 ± 0,21
25	91,42 ± 3,54	53,67 ± 3,9
12,5	68,17 ± 1,12	24,79 ± 0,29
6,25	64,16 ± 0,69	7,92 ± 0,14
3,125	52,66 ± 1,49	6,40 ± 1,2
IC 50	3.48	18.19

The absorbance data obtained was then calculated the percentage of inhibition and processed with the SPSS statistical test. The Shapiro-Wilk normality test that was carried out showed that the data had a normal distribution because p> 0.05.

With the Levene homogeneity test carried out simultaneously with the t-test on SPSS having p>0.005, the analysis that is considered is the analysis on the homogeneous part. The overall analysis considered shows that all pairs of t-test test pairs are significant or significantly different due to the p value <0.05.

IC50 Value of Ethanol Extract of *Mangifera quadrifida* and Acarbose

The IC50 value is the sample concentration which required to inhibit 50% of the activity of the α-glucosidase enzyme. This value is obtained from a linear regression graph on the log of sample concentration on the x-axis with the percentage of inhibition on the y-axis. On the linear graph, the equation of the line y = ax + b is formed. The variable y describes the percentage of inhibition that can be replaced by 50 with the aim of getting the IC value of 50 by calculating from 10 to the power of x.

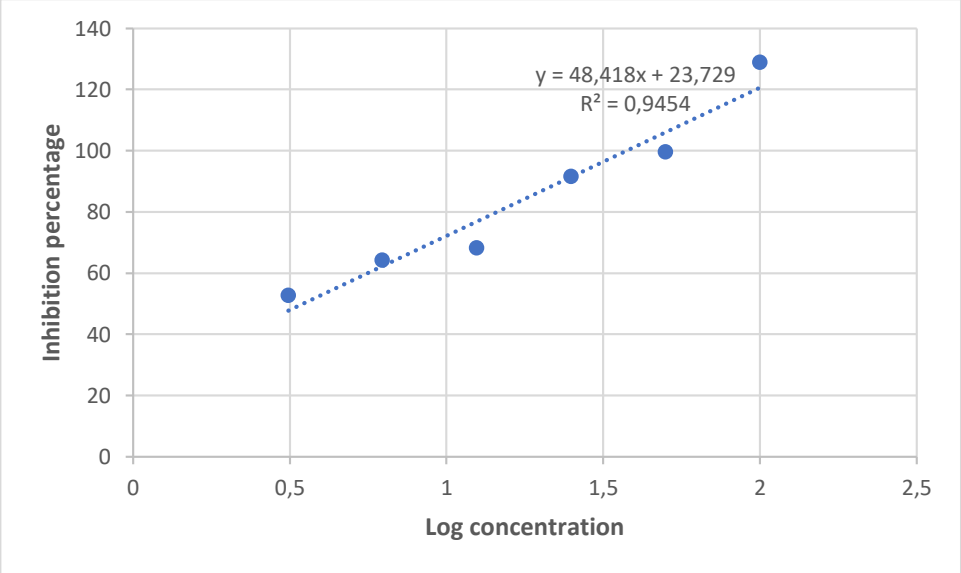

Figure 1. Log concentration linear regression Graph of alpha-glucosidase inhibition activity of Acarbose

In the log graph of the concentration of the mean percentage of α-Glucosidase inhibition, the equation is in the form of y= 48.418x + 23,729. From the calculation, it was concluded that the IC_{50} value of acarbose is 3.488 ppm.

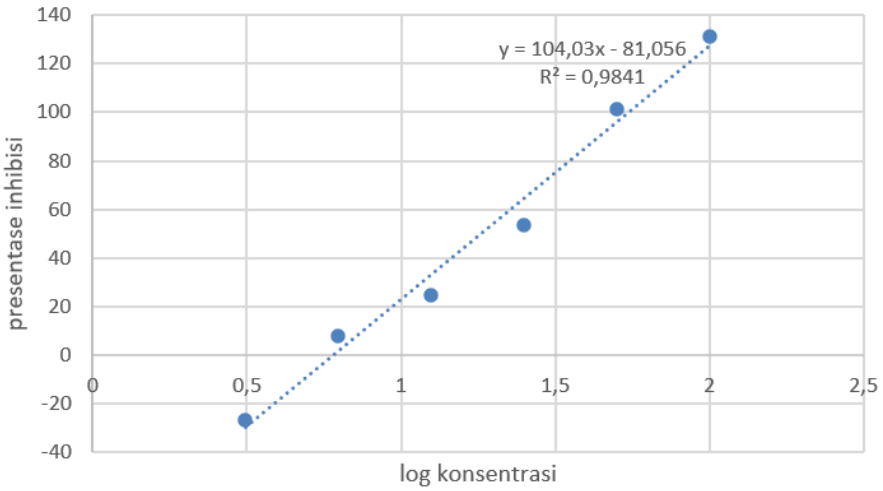

Figure 2. Log concentration linear regression Graph of α-glucosidase inhibition activity of ethanol extract of *Mangifera quadrifida*

In the log graph of the concentration of the mean percentage of -glucosidase inhibition, it is found that the equation is in the form of y= 104.03x-81.056. From the calculation, it was concluded that the IC50 of *Mangifera quadrifida* ethanol extract value is 18.19 ppm.

Mangifera quadrifida fruit ethanol extract has α-glucosidase inhibitory activity by in-vitro which less than akarbosa which has very high inhibitory activity. Nevertheless, the extract has inhibitory activity with active level, so that can considered in research about anti-diabetic in the future. As an anti-diabetic the classification of IC50 considered as very active if it's less than 11 ppm, active if 11-100 ppm, and not active if >100 ppm.

4. Materials and Methods

4.1. Mangifera quadrifida Exctraction

Mangifera quadrifida was cut and wind dried, and then grinded into fine powder. *Mangifera quadrifida* powder then macerated by ethanol for 3x24 hours. Next, filtered use filter paper and evaporated use rotary evaporator.

4.2. Phytochemical Screening Test

Tannin, flavonoid, triterpenoid, saponin, and glycoside were phytochemical that have been tested.

4.2.1 Flavonoid Screening Test

Amount of 1 mL *Mangifera quadrifida* solution extract would be evaporated and treated by acetone. Then, added boric acid powder and oxalic acid powder. Next, solution was mixed with 10mL ether and measured use UV 366 nm wavelength. Flavonoid compound would be proven by yellow color.

4.2.2 Tannin Screening Test

Amount of 1 mL *Mangifera quadrifida* solution extract was mixed with iron (III) chloride 10%. Tannin would be proven by dark blue or black-green colored solution.

4.2.3 Triterpenoid Screening Test

Amount of 2 mL *Mangifera quadrifida* solution extract would be evaporated and dissolved in 0.5 mL of chloroform. Then, added 0.5 mL anhydrate acetic acid and 2 mL concentrated sulfuric acid through test tube wall. Triterpenoid would be proven by existence of brown or violet ring on solution border.

4.2.4 Saponin Screening Test

Amount of 10 mL *Mangifera quadrifida* solution extract was shaken for 10 seconds and let it for 10 seconds. Then, foam that formed by saponin (1-10 cm) was observed. Foam would last about 10 minutes and would not disappear even added with 1 drop of HCL 2N.

4.2.5 Glycoside Screening Test

Amount of 0.1 mL *Mangifera quadrifida* solution extract was evaporated by water bath. The leftovers was dissolved back in 5 mL anhydrate acetic acid. Glycoside would be

proven by green or blue colored when solution was added by 10 drops of concentrated sulfuric acid.

4.3. Chromatography

Thin layer chromatography is a chromatography technique used to separate mixtures with stationary phase (silica) that was coated on the aluminium plate or glass and mobile phase that was mixture of non-polar and polar solvent. Dissolved sample was pasted by capillary pipes on the stationary phase thin plate surface, then was straightened on the vessel which contain mobile phase. Because of capillary action. Mobile phase would drawn up through the plate and brought components of sample.

Compound that separated in the TLC, was identified by UV ray on 254 or 366 nm wavelength. It also can be identified by staining reaction with iodine. Compound spot on TLC plate can be calculated for Retention factor (Rf), by divided the distance the compound traveled from the original position by the distance the solvent travelled from original position. Retention factor can be used for identify compound groups which contain in Mangifera qudrifida ethanol extract.

4.4. α-glucosidase Inhibitory Activity Assay

Amount of 200 μL of α-glucosidase enzyme was mixed with 200 μL of samples that contain plant extract (0.06-1.18 mg/mL) or acarbose solution (0.001-0.118 mg/mL) solution and 1 mL phosphate buffer. Samples was preincubated at 37°C for 10 min. Then, added and mixed 300 μL 10 mM p-NPG and incubated further at 37° C for 40 min. The reaction was stopped by adding 3 mL Na2CO3 (100 mM). The absorbance of the released p-nitrophenol was measured at 405 nm wavelength. Buffer solution was used as a blank. Test tube which contain solution without extract was used as a control. The results were express as percentage inhibition, which calculated using the formula :

$$\alpha\text{-}glucosidase\ inhibitory\ activity - glucosidase\ (\%) = \frac{(\mathbf{Abs\ C - Abs\ S})}{(\mathbf{Abs\ C})} \times 100$$

Where, Abs C was control absorbance (100% enzyme activity) and Abs S was test substance absorbance.

In this study, the independent variable was *Mangifera quadrifida* extract. Meanwhile the dependent variable was chemical substance on ethanol extract of *Mangifera quadrifida* and α-glucosidase inhibitory activity. Then, confounding variable were *Mangifera quadrifida* plant environment, solvent properties, temperature, and pH of enzyme environment. Research variable was independent and dependent variable.

4.5. Data result and present

The result in this study would be processed with software (Microsoft Excel and SPSS ver.24). Microsoft excel was used to show relation graph between extract concentration on enzyme inhibition percentage. Microsoft excel also used to count the linear regression and IC¬50. Results that processed by Microsoft excel also processed by SPSS ver. 24 or R 4.0.5 ver. Analysis of α-glucosidase inhibition could be obtained by data normality test with Shapiro-Wilk, it because samples per group was less than 30. Parametric test would be processed using independent t-test method if distributed as normal. If data would not distributed as normal, then would be processed with non-parametric test with Mann-Whitney U method. Result considered statistically if p value < 0.055.

5. Conclusions

Chemical substance which contained in *Mangifera quadrifida* ethanol extract are flavonoid, tannin, glycosidase, saponnin, and triterpenoid that have many benefit, one of them is as anti-diabetic. α-glucosidase inhibitory activity is founded on *Mangifera quadrifida* ethanol extract that active (IC50 = 18.19 ppm) with positive control (akarbose) (IC50 = 3.488 ppm) as anti-diabetic. *Mangifera quadrifida* fruit ethanol extract has α-glucosidase inhibitory activity by in-vitro which less than akarbosa which has very high inhibitory activity. Nevertheless, the extract has inhibitory activity with active level, so that can considered in research about anti-diabetic in the future.

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