

Phytochemical and Anti-diabetic Activity Studies of n-Hexane, Ethyl acetate and Methanol Extracts of Matoa (*Pometia pinnata*) Fruit Peel Using Alpha-Glucosidase Enzyme

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Abstract—This study focused on the phytochemical and anti-diabetes activity of n-Hexane, Ethyl acetate, and Methanol Extracts of Matoa (*Pometia pinnata*) fruit peel from West Kalimantan Indonesia using Alpha-Glucosidase enzyme. The experiment consisted of three stages, which are 1) extraction of the Matoa (*Pometia pinnata*) peel using n-Hexane, Ethyl acetate and Methanol; 2) Phytochemical test; and 3) anti-diabetic activity test using Alpha-Glucosidase enzyme. The most effective extraction result of matoa fruit peel is using n-hexane solvent. The phytochemical test result showed that n-Hexane extract of Matoa fruit peel contained tannins, phenols, saponins and, little terpenoids; Ethyl acetate extract contains flavonoids, tannins and phenols; while methanol extract contains flavonoids, tannins, phenols and, saponins. The best anti-diabetic activity test results were obtained from ethyl acetate extract. It can be concluded that compounds contained in the Ethyl acetate extract of Matoa fruit peel potentially become s an active anti-diabetic substance.

Keywords—phytochemical, anti-diabetic, fruit peel of matoa (*pometia pinnata*), alpha-glucosidase enzyme

I. INTRODUCTION

The World Health Organization (WHO) estimates that the number of type 2 Diabetes Mellitus (DM) sufferers in Indonesia will increase significantly to 21.3 million by the next 2030. This contributes to the prediction that Indonesia will experience potential losses of up to Rp 71 thousand trillion due to non-communicable diseases in the 2015-2035 period. Besides, Evidence & Analytics (health research institute based in Manchester, England) state that the loss is an accumulation of medical expenses and variable expenses as a result of the disease, including the loss of patient productivity in working age [1].

The increasing number of diabetes mellitus sufferers encourages researchers to find herbal treatments by utilizing various types of medicinal plants that contain active ingredients

that can reduce blood sugar levels. One of the plants that have the potential for the treatment of Diabetes Mellitus is Matoa (*Pometia pinnata*). Indonesia has plants from the Sapindaceae family which are widely spread in various regions and among them have been used as daily necessities and traditional medicines. Matoa Plant (*Pometia pinnata*) is a typical native plant from Papua but has spread over the territory of Indonesia covering Sumatra, Java, Kalimantan, Sulawesi, Sumbawa Island (NTB), and Maluku.

Several previous studies on antidiabetic have been carried out, such as Pare plants (*Momordica charantia*) [2], use Pandan Wangi leaves (*Pandanus amaryllifolius*, Roxb.) and other researchers use the Matoa plant (*Pometia pinnata*) [3]. The research utilizing matoa as an antidiabetic was carried out through the extraction of matoa peel using a maceration technique using 3 solvents, namely acetone, ethanol, and aquades [4]. A comparison of total phenolic contained in seeds, fruit flesh, matoa peel from Indonesia has also been studied [5]. Besides, the evaluation of DPPH Matoa free radical activity (*Pometia pinnata*) from Indonesia has been studied [6], as well as phytochemicals and total phenolic content of methanol extract from Matoa (*Pometia pinnata*) from Papua, Indonesia [7].

Based on the results of previous studies it has been shown that the peel of matoa contains tannins, saponins, and alkaloids [4]. The compounds contained in the peel of the matoa has the potential to be able to neutralize the sugar content in the blood. To be able to obtain substances that function to cure diabetes, a study of the activity of antidiabetic compounds of matoa peel extract (*Pometia pinnata*) is needed. One of the test methods for the activity of antidiabetic compounds is using the Alpha-Glucosidase enzyme [8,9].

The purpose of the study is to determine the phytochemical and activity of antidiabetic compounds of the extract of matoa (*Pometia pinnata*) fruit peel. The activity of antidiabetic

compounds of matoa fruit peel was tested using the Alpha-glucosidase enzyme. This research is expected to provide information about the phytochemical and potential of matoa fruit peel extract as anti-diabetic.

II. MATERIALS AND METHODS

This study was divided into three stages. The stages are 1) extraction of matoa fruit peel using three different solvents (n-Hexane, Ethyl acetate, and Methanol); 2) Phytochemical test; and 3) Anti-diabetic activity test using an Alpha-Glucosidase enzyme.

A. Extraction of Matoa Fruit Peel

Matoa fruit from West Kalimantan Indonesia was collected and peeled, then the outer shell was separated. Then the matoa fruit peel was dried by aerating at room temperature. Fifty grams of matoa fruit peel *Simplicia* were extracted with a solvent ratio of 1:10 (w / v). The solvents used were n-Hexane, Ethyl acetate, and Methanol. Extraction was carried out for 3x24 hours by maceration. The results of maceration were filtered, then evaporated with a rotary evaporator to obtain a dry extract at a temperature of 50°C. The residue obtained from the first bath was macerated again with the same treatment, the results were filtered and evaporated again. Furthermore, the same treatment was carried out for Ethyl acetate and Methanol solvents. The extracted content can be calculated by this formula below.

$$\text{Content of extract (\%)} = \left(\frac{\text{Weight of extract}}{\text{Weight of Simplicia}} \right) \times 100\% \quad (1)$$

B. Phytochemical Test of Matoa Fruit Peel Extract

The phytochemical test of matoa fruit peel extract was carried out by identifying alkaloid compounds, flavonoids, triterpenoids, saponins, and tannins according to the method of Edoega et al [10,11].

C. Antidiabetic Potential Test of Matoa Fruit Peel Extract Using Alpha-Glucosidase Enzyme

1) *Extract preparation*: The sample was weighed as much as 2 mg and dissolved with 200 µL dimethyl sulfoxide (DMSO). The sample was piped 5 µL and diluted with 200 µL phosphate buffer pH 7. Samples were made in various concentrations namely 3.125 ppm, 6.25 ppm, 12.50 ppm, and 25 ppm.

2) *Enzyme preparation*: The enzyme solution was made by dissolving 1.0 mg α-glucosidase in 100 mL pH 7 phosphate buffer containing 200 mg of serum albumin bovine. Before use, 1 milliliter of the enzyme solution was diluted 25 times with a pH 7 phosphate buffer.

3) *Blank testing*: Five µL of DMSO added with 495 µL of phosphate buffer pH 7 and 250 µL 20 mM p-Nitrofenyl α-D-glucopiranoside (PNP), Blanko was incubated for 5 minutes at 37°C. In the sample, 250 µL of the enzyme was diluted, the

sample was incubated again for 15 minutes at 37°C. After the incubation period, 1000 µL 200 mM Na₂CO₃ was added. The samples were measured for absorbance using a UV-Vis Spectrophotometer at a wavelength of 400 nm [12].

4) *Sample testing*: A total of 5 µL samples were added with 495 µL phosphate buffer pH 7 and 250 µL 20 mM p-Nitrofenyl α-D-glucopiranoside (PNP), the samples were incubated for 5 minutes at 37°C. In the sample added 250 µL of the enzyme that had been diluted, the sample was incubated again for 15 minutes at 37°C. After the incubation period was completed 1000 µL 200 mM Na₂CO₃ was added. The samples were measured for absorbance using a UV-Vis Spectrophotometer at a wavelength of 400 nm.

5) *Sample testing without enzymes*: As much as 5 µL samples were added with 495 µL phosphate buffer pH 7 and 250 µL 20 mM p-Nitrofenyl α-D-glucopiranoside (PNP), the samples were incubated for 5 minutes at 37 ° C. In the sample added 250 µL phosphate, buffer pH 7, 100 milliliters, the sample was incubated again for 15 minutes at 37°C. After the incubation period was completed 1000 µL 200 mM Na₂CO₃ was added. Samples were measured absorbance with a UV-Vis Spectrophotometer at a wavelength of 400 nm. [11].

6) *Calculation of Percentage Inhibition and IC₅₀*: Percentage Inhibition and IC₅₀ can be calculated with the formula below.

$$\% \text{ Inhibition is calculated by formula} = \frac{[\text{Blank Absorbance} - \text{Sample Absorbance}] / \text{Blank Absorbance}}{\text{Blank Absorbance}} \times 100\% \quad (2)$$

IC₅₀ was calculated using a linear regression equation, the concentration of the sample as the x-axis, and % inhibition as the y-axis. From equation = a + bx IC₅₀ can be calculated with IC₅₀ (50 - a): b.

III. RESULTS AND DISCUSSION

A. Extraction Results

The extraction of matoa peel using n-Hexane, Ethyl acetate, and Methanol solvents can be seen in Table 1.

TABLE I. EXTRACTION OF MATOFA FRUIT PEEL RESULT

Extraction Results (%)		
<i>n-Hexane</i>	<i>Ethyl acetate</i>	<i>Methanol</i>
54.15 ± 0.07	7.12 ± 0.05	14.29 ± 0.07

From Table 1, it can be seen that the most extracts were found in extraction using n-Hexane and the lowest was obtained from Methanol extract. The most effective extraction result of matoa fruit peel is using n-Hexane solvents.

B. Phytochemical Test

The phytochemical test is an initial test carried out to determine the components or chemical compounds contained in natural ingredients. This test is carried out qualitatively using several types of tests and reagents. The phytochemical screening test consisting of alkaloids, flavonoids, tannins, phenols, saponins, and terpenoids in matoa fruit peel extracts was carried out using three different solvents, namely n-Hexane, Ethyl acetate, and Methanol. The three solvents have different polarity properties, namely, n-Hexane is non-polar, Ethyl acetate is semi-polar, and Methanol is polar. The results of the matoa fruit phytochemical test can be seen in Table II.

Based on Table 2, it is known that the n-Hexane extract of matoa fruit peel contains tannins, phenols, saponins, and slightly terpenoids. Ethyl acetate extract contains flavonoids, tannins, and phenols. While methanol extract contains flavonoids, tannins, phenols, and saponins. The test results showed that not all extracts contain flavonoids.

TABLE II. PHYTOCHEMICAL TEST RESULTS OF MATOA FRUIT PEEL EXTRACT

Phytochemical Test	Solvent		
	n-Hexane	Ethyl Acetate	Methanol
Alkaloid :			
Meyer's	-	-	-
Dragendorff's	-	-	-
Wagner's	-	-	-
Flavonoid	-	++	+
Tanin	+++	+++	+++
Phenol	+++	+++	+++
Saponin	++++	-	+++
Terpenoid	+	-	-

Several types of chemical compounds are extracted with the level of the polarity of compounds contained in natural materials.

Phytochemical compounds have been studied and are known to have health benefits. Tannin is antibacterial, flavonoids are polar so that they can easily penetrate the peptidoglycan layer, which is also polar, and saponins can lyse bacterial cell walls when interacting with bacterial walls [13].

C. Test Results of Antidiabetic Activity

Based on the antidiabetic activity test standard quercetin at a concentration of 10; 7.5; 5; 2.5 and 1 µg / mL, it is obtained as percentage inhibition as shown in Table 3.

TABLE III. ANTIDIABETIC ACTIVITY TEST RESULTS

Sample	Concentration (µg/mL)	Inhibition (%)	IC50 (µg/mL)
Quercetin	1	7.4	6.0 ± 0.1
	2.5	27	
	5	45	
	7.5	61	
	10	78	
n-Hexane extract of Matoa fruit peel	10	6.51	101 ± 3
	25	9.98	
	50	17.94	
	100	53.11	
Ethyl Acetate Extract of Matoa fruit peel	10	19	57 ± 3
	25	33	
	50	50	
	100	73	
Methanol extract of Matoa fruit peel	10	16.52	116 ± 4
	25	17.86	
	50	19.37	
	100	46.16	

Based on Table 3 above, it can be seen that the results of extracting matoa peel have the highest value of antidiabetic activity with an IC₅₀ value of 116 ± 4 (µg / mL). The results of the test values for antidiabetic activity (IC₅₀) from the extract of matoa fruit peel using n-Hexane, Ethyl acetate, and Methanol, respectively (101 ± 3) µg / mL; (57 ± 3) µg / mL and (116 ± 4) µg / mL. The value of the antidiabetic activity of the extract of the matoa fruit peel uses n-Hexane, Ethyl acetate, and Methanol to increase with increasing concentration. The highest anti-diabetic activity was in ethyl acetate extract, but the activity was still greater than that of quercetin as standard, namely IC₅₀ 6.0 ± 0.1 µg / mL. Quercetin is a compound that is usually used as a standard in antidiabetic testing because it has a high ability to inhibit the activity of the alpha-glucosidase enzyme [14,15].

Percentage of inhibition of α-glucosidase from quercetin standards can be seen in Figure 1, while percentage inhibition of the results of extraction of fruit peel using n-Hexane, Ethyl acetate, and Methanol can be seen in Figures 2, 3, and 4.

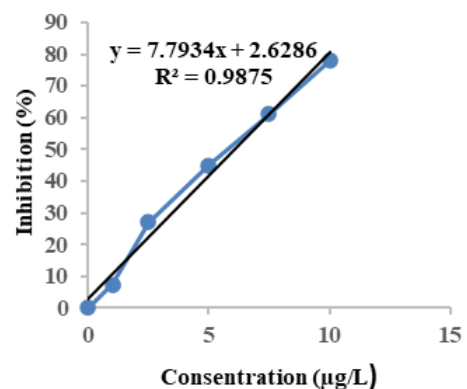


Fig. 1. Percentage of Quercetin Standard α-Glucosidase enzyme inhibition.

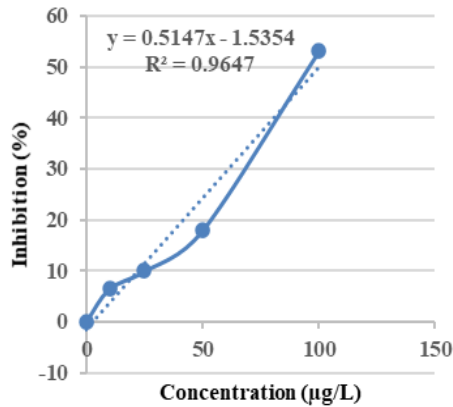


Fig. 2. Percentage of inhibition of the α -glucosidase enzyme from n-hexane extract of matoa fruit peel.

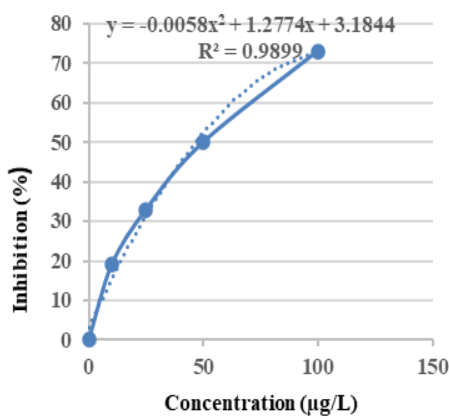


Fig. 3. Percentage of inhibition of the α -glucosidase enzyme from ethyl acetate extract of matoa fruit peel.

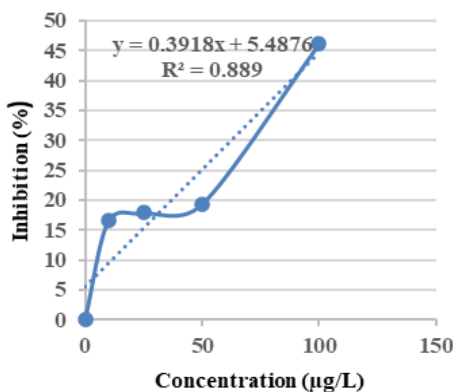


Fig. 4. Percentage of inhibition of the α -glucosidase enzyme from methanol extract of matoa fruit peel.

Based on the antidiabetic test used α -Glucosidase inhibition test, quercetin standard solution at a concentration of 10, 7.5, 5, 2.5, and 1 $\mu\text{g/mL}$ had percentage inhibition of 78%, 61%, 45%, 27%, and 7.4% respectively (Fig.1). The n-Hexane extracts of *Pometia pinnata* fruit peel at a concentration of 100, 50, 25, dan 10 $\mu\text{g/mL}$ had a percentage inhibition of 53.11%, 17.94%, 9.98%, and 6.51% respectively (Fig. 2). Ethyl acetate extracts of *Pometia pinnata* fruit peel at the same concentration had a

percentage inhibition of 73%, 50%, 33%, 19% respectively (Fig.3). Extraction using Methanol of *Pometia pinnata* fruit peel at the same concentration had a percentage inhibition of 46.16%, 19.37%, 17.86%, and 16.52% respectively (Fig.4).

IV. CONCLUSION

The result of extraction using n-Hexane, Ethyl acetate, and Methanol solvent were respectively ($54.15 \pm 0.07\%$); ($7.12 \pm 0.05\%$); and ($14.29 \pm 0.07\%$). The most effective extraction result of matoa fruit peel is using n-Hexane solvent. The phytochemical showed that n-Hexane extract of matoa fruit peel contains tannins, phenols, saponins, and slightly terpenoids; Ethyl acetate extract contains flavonoids, tannins, and phenols; while Methanol extract contains flavonoids, tannins, phenols, and saponins. The phytochemical screening of the matoa fruit peel plays an important role in pharmaceutical studies especially discovering new potential drugs for the treatment of diabetic diseases. The test values for anti-diabetic activity (IC_{50}) from the extract of matoa peel using n-Hexane, Ethyl acetate, and Methanol were respectively (101 ± 3) $\mu\text{g/mL}$; (57 ± 3) $\mu\text{g/mL}$, and (116 ± 4) $\mu\text{g/mL}$. The best anti-diabetic activity test was obtained from the Ethyl acetate extract of matoa fruit peel. It can be concluded that compounds contained in the Ethyl acetate extract of matoa fruit peel from West Kalimantan Indonesia potentially become an active anti-diabetic substance.

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