

## ANTIHYPERGLYCEMIC ACTIVITY OF THE MAHKOTA DEWA [*Phaleria macrocarpa* (Scheff.) Boerl.] LEAF EXTRACTS AS AN ALPHA-GLUCOSIDASE INHIBITOR

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### Abstract

Alpha-glucosidase (EC 3.2.1.20) is a carbohydrase that catalyzes the liberation of  $\alpha$ -glucose from the nonreducing end of the diet carbohydrate. In diabetic patients, inhibition of these enzymes causes the restraint of glucose absorption and decreases the postprandial hyperglycemia. The purpose of this research is to study the antihyperglycemic activity of *mahkota dewa* [*Phaleria macrocarpa* (Scheff.) Boerl.] leaf extracts by inhibition test to alpha glucosidase enzyme. This research was conducted in three steps: fractionation and extraction samples with methanol, ethyl acetate, *n*-butanol, and water, followed by phytochemistry screening and alpha-glucosidase inhibition test. The alpha-glucosidase inhibition test was performed by using alpha-glucosidase enzyme and *p*-nitrophenyl  $\alpha$ -D-glucopyranoside as a substrate. The result of phytochemistry screening showed that *Mahkota dewa* leaves contain class of phenolics, thanins, flavonoids, alkaloids, and carbohydrates. The result of alpha-glucosidase inhibition test showed that ethyl acetate fraction extract had the highest inhibition activity with inhibition percentage at 50 ppm for old leaves which is 55.04% and for young leaves which is 56.92%. At 50 ppm, inhibition activity from the methanol extract and boiled water extract of old leaves is higher than that of young leaves with inhibition percentage of old leaves methanol extract which is 14.25% and 10.97% for young leaves and for old leaves; boiled water extract is 10.32% and 6.85% for young leaves. For *n*-butanol fraction extract, inhibition activity of young leaf extract (14.26%) is higher than old leaf extract (9.49%).

*Keywords:* alpha-glucosidase inhibitor, diabetes mellitus type 2, mahkota dewa, *Phaleria macrocarpa*

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### Introduction

Diabetes Mellitus (DM) is a group of symptoms that appears on someone showed by the content of the above normal glucose in one's blood (hiperglycemia) caused by insufficient insulin in the body.<sup>1</sup> The occurrence phenomenon of DM disease increases from year to year. According to WHO survey, Indonesia places in the fourth position in the world largest number of DM patients after India, China, and the United States of America. The prevalence of DM in Indonesia is 8.6% from the total population. Based on the prevalence, the number of Indonesian DM patients in 1995 reached up to 4.5 million people, in 2001 it reached 5.6 million people, and in 2025 it is predicted that it will reach 12.4 million people.<sup>2</sup>

DM medication can be performed with insulin injection and modern medicine, such as oral antidiabetic which

consists of sulfonilurea, biguanid, thiazolidinedion, and alpha-glucosidase inhibitor. Alpha-glucosidase inhibitor is used to medicate DM Type 2. This type of medicine does not increase insulin secretion. Antihyperglycemic exertion of alpha-glucosidase inhibitor derives from reversible inhibition, competitive to intestinal carbohydrate digestion enzymes, such as pancreatic alpha-amylase,  $\alpha(1\rightarrow6)$  glucosidase (isomaltase), sucrase, and maltase. These enzymes hydrolyze dietary carbohydrates to glucose. In diabetic patients, inhibition of these enzymes causes inhibition of glucose absorption and decreases the post prandial hyperglycemia.<sup>3</sup>

*Mahkota dewa* (*Phaleria macrocarpa* (Scheff.) Boerl.) is originated from Papua (Indonesia) and has been used traditionally to medicate DM and other diseases, such as cancer, lever, rheumatic, gout, kidney, heart disease, hipertension, eczema, acne, and injury caused by insect bites.<sup>4</sup> The existence of scientific evidence concerning the

hypoglycemic effect of this plant with alpha-glucosidase inhibition test is very important in order to make this Indonesian plant become a standard herb and as a phytopharmaca supply which is medically accountable.

The purposes of this research are to perform hyperglycemic activity test from the young and old leaves of *mahkota dewa* with alpha-glucosidase enzyme inhibition test and to find out alpha-glucosidase enzyme inhibition effectivity from various young and old leaf extracts of *mahkota dewa*.

**Methods**

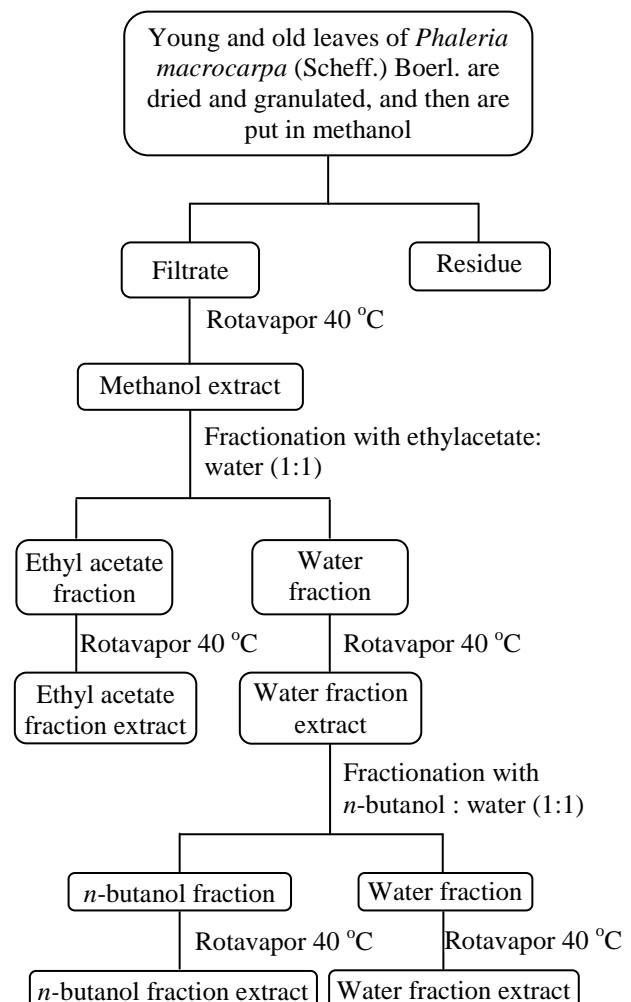
This research was performed in three steps. The first step was fractionated and extracted samples from the young and old leaves of *mahkota dewa* with methanol, ethyl acetate, *n*-butanol and water as a solvent. The second step was phytochemistry test and the third step was alpha-glucosidase inhibition test on various leaf extracts.

**Fractination and Extraction.** 200 gram of *mahkota dewa* old leaf powder is put in 1.5 litre methanol solvent for 4 days at a room temperature with 4 times replications, and then the mixture is filtered and evaporated by rotary evaporator at 40 °C until the concentrated methanol extract is obtained. The methanol extract of young leaves is obtained with the same way as old leaf methanol extract. After that, methanol extract is fractionated with the mixture of water and ethyl acetate solvent (1:1) to obtain ethyl acetate fraction and water fraction. Water fraction is refractionated by *n*-butanol and obtaining *n*-butanol fraction and water fraction. Each fraction is concentrated by rotary evaporator at 40 °C until ethyl acetate fraction extract is obtained. Fractionation and extraction of samples could be seen in Figure 1. In this research, obtaining water extract can be done by boilling the leaves of *mahkota dewa*, besides by fractionation.

**Phytochemistry Test.** In this research we performed phytochemistry test which consists of alkaloid test with Dragendorff reagent, flavonoid test with magnesium powder and concentrated HCl, phenol test and thanin test with ferrous (III) chloride, carbohydrate test with Molish reagent and concentrated H<sub>2</sub>SO<sub>4</sub>, and Biuret test with NaOH dan CuSO<sub>4</sub>.

**Alpha-Glukosidase Inhibition Test** Enzyme solution is made by dissolving 1,0 mg alpha-glucosidase in 100 mL phosphat buffer (pH 7,0) that contains 200 mg bovin albumin serum.<sup>5,6</sup> Before it is used, 1 mL of the enzyme solution is diluted 25 times with phosphat buffer (pH 7.0) that contains 200 mg bovin albumin serum. The mixture contains 250 µL 20 mM *p*-nitrofenil α-D-glukopiranoside as a substrate, 490 µl 100 mM phosphat buffer (pH 7,0) and 10 µL solution sample in DMSO. After the mixture is incubated at 37 °C for 5 minutes,

250 µL enzyme solution is added and incubated for 15 minutes. Enzymatic reaction is stopped by adding 1000 µL 200 mM sodium carbonate, and the the absorbance of *p*-nitrophenol obtained is read at 400 nm. The complete enzyme reaction system for one sample with 2 mL total volume can be seen in Table 1.



**Figure 1. Fractionation and Extraction Chart of the Mahkota dewa Leaves**

**Tabel 1. Enzym Reaction System for One Sample with 2 mL Total Volume**

	Blank µL	control µL	So µL	S1 µL
Sample	-	-	10	10
DMSO	10	10	-	-
Buffer	490	490	490	490
Substrate	250	250	250	250
Incubation in waterbath at 37 °C, 5 minutes				
Buffer	250	-	250	-
Enzyme	-	250	-	250
Incubation in waterbath at 37 °C, 15 minutes				
Na <sub>2</sub> CO <sub>3</sub>	1000	1000	1000	1000

Samples are methanol extract, ethyl acetate fraction extract, *n*-butanol fraction extract, water fraction extract and water boiled extract of *mahkota dewa* leaves with 1%, 0.5%, 0.25% and 0.125% concentration variation and DMSO as a solvent. For each extract we performed alpha-glucosidase inhibition test with 3 times repetition (triplo). The inhibition percentage could be calculated with equation:

$$[(C - S) / C] \times 100 \quad (1)$$

In which S = sample absorbance (S1-S<sub>0</sub>), S1 = sample absorbance with enzyme addition, S<sub>0</sub> = sample absorbance without enzyme addition, C = absorbance control (DMSO) without samples (control-blank).

## Results and Discussion

**Phytochemistry Test.** In phytochemistry test, methanol extract, ethyl acetate fraction extract, *n*-butanol fraction extract, water fraction extract, and boiled water extract of young and old *mahkota dewa* leaves could identify the existence of phenol, thanin, flavonoid, alkaloid, and carbohydrate.

**Alpha-Glucosidase Inhibition Test.** The alpha-glucosidase inhibition test is performed to determine the antihyperglycemic activity of all extracts. In this experiment, alpha-glucosidase enzyme will hydrolyse *p*-nitrophenyl  $\alpha$ -D-glucopyranoside to become *p*-nitrophenol with yellow color and glucose with the following reaction (Figure 2).

Enzyme activity is measured based on *p*-nitrophenyl absorbance result in yellow color.<sup>7</sup>

Figure 3 and 4 illustrate inhibition percentage to alpha-glucosidase enzyme of various extracts from the young and old leaves of *mahkota dewa* at 6.25 ppm, 12.5 ppm, 25 ppm, and 50 ppm.

Figure 3 illustrates that ethyl acetate fraction extract has the highest inhibition activity in almost all variations of the concentration tested (55.04% at 50 ppm, 32.63% at 25 ppm, 19.91% at 12.5 ppm) except at 6.25 ppm (3.8%). At 50 ppm, methanol extract has inhibition activity 14.25% followed by boiled water extract (10.32%) and *n*-butanol extract (9.49%). At 25 ppm and 12.5 ppm, *n*-butanol fraction extract has inhibition activity 8.25% (25 ppm) and 7.99% (12.5 ppm) which is higher than boiled water extract (6.81% at 25 ppm and 5.52% at 12.5 ppm), methanol extract (5.52% at 25 ppm and 1.61% at 12.5 ppm). At 6.25 ppm, *n*-butanol fraction extract still has inhibition activity (7.43%) higher than boiled water extract (5.14%) and methanol extract (0.46%). Water fraction extract does not have inhibition activity in almost all of the concentration tested, except at 50 ppm (1.53%).

Figure 4 illustrates that ethyl acetate fraction extract has the highest inhibition activity in almost all concentration tested followed by *n*-butanol fraction extract, methanol extract, and boiled water extract. At 50 ppm and 25 ppm ethyl acetate fraction extract has the highest inhibition activity (56.92% at 50 ppm and 27.1% at 25 ppm) followed by *n*-butanol fraction extract (14.26% at 50 ppm and 13.32% at 25 ppm), methanol extract (10.97% at 50 ppm and 7.56% at 25 ppm) and boiled water extract (6.85% at 50 ppm, 6.08% at 25 ppm).

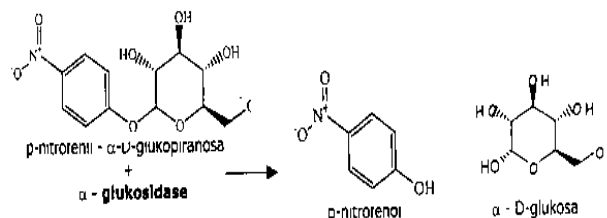


Figure 2.  $\alpha$ -Glucosidase and *p*-nitrophenyl  $\alpha$ -D-Glucopyranoside Enzymatic Reaction Equation

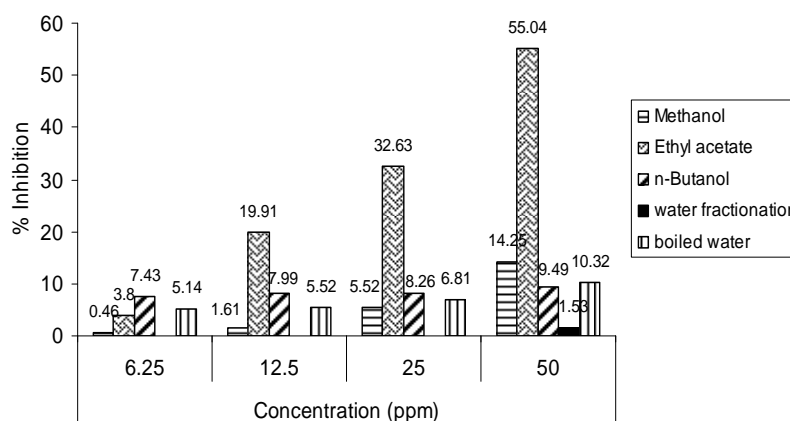
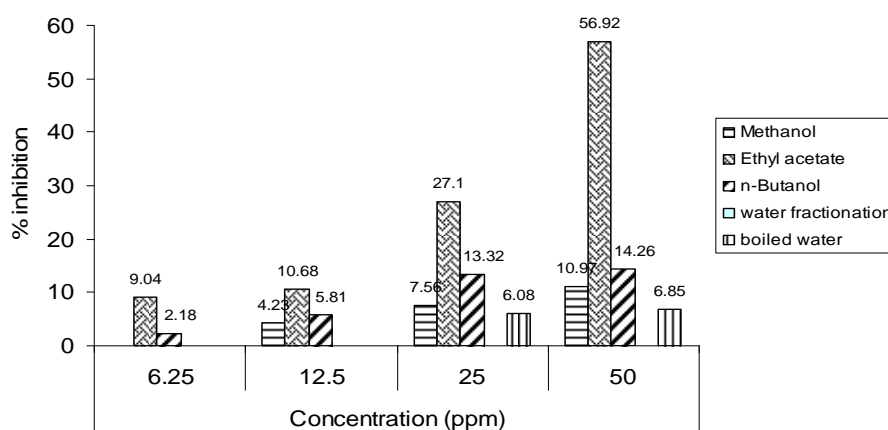
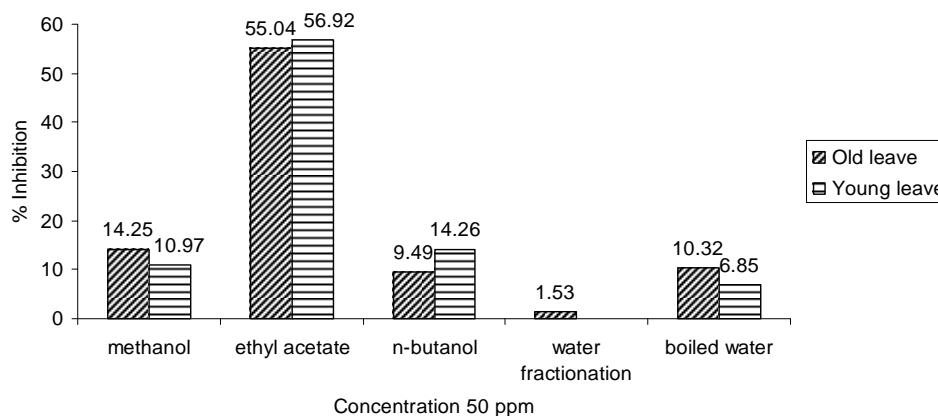


Figure 3. Inhibition Percentage to Alpha-Glucosidase Enzyme of Methanol Extract, Ethyl Acetate Fraction Extract, *n*-butanol Fraction Extract, Water Fraction Extract, and Boiled Water Extract of *Mahkota dewa* Old Leaves



**Figure 4. Inhibition Percentage to Alpha-glucosidase Enzyme of Methanol Extract, Ethyl Acetate Fraction Extract, n-butanol Fraction Extract, Water Fraction Extract, and Boiled Water Extract of *Mahkota dewa* Young Leaves**



**Figure 5. Inhibition Activity Comparison of the Young and Old Leaves of *Mahkota dewa* at 50 ppm**

ppm). At 12.5 ppm, ethyl acetate fraction extract still has the highest inhibition activity (10.68%) followed by n-butanol extract (5.81%) and methanol extract (4.23%). Meanwhile, boiled water extract does not have inhibition activity. At 6.25 ppm concentration, only ethyl acetate fraction extract (9.04%) and n-butanol fraction extract (2.18%) still have inhibition activity. Water fraction extract does not have inhibition activity in all concentration tested.

Figure 5 illustrates the comparison of inhibition activity from various extracts of the old and young leaves at 50 ppm. The old leaves from methanol extract and boiled water extract have higher inhibition activity than young leaves where inhibition percentage of the methanol extract is 14.25% for old leaves and 10.97% for young leaf, and boiled water extract for old leaves is 10.32% and 6.85% for young leaves. For ethyl acetate fraction extract, the inhibition activity of old leaves (55.04%) is different from young leaf extract (56.92%). For n-butanol fraction extract, the inhibition activity of young leaf extract (14.26%) is higher than old leaf extract (9.49%).

The presence of inhibition to alpha-glucosidase activity of methanol extract, ethyl acetate fraction extract, n-butanol fraction extract and boiled water extract of young and old leaves could be caused by the presence of carbohydrate, which is suspected to be the competitive inhibitor for alpha-glucosidase enzyme. This is appropriate with the substrate of alpha-glucosidase which is food carbohydrate, such as starch and glycogen. Besides that, this prediction is based on the type of alpha-glucosidase inhibitor drugs, such as Acarbose and Myglitol which are carbohydrate compound. Acarbose is an oligosaccharide which is obtained from fermentation processes of a microorganism, *Actinoplanes utahensis*, and is chemically known as O-4,6-dideoxy-4-[[[(1 S,4 R, 5 S, 6 S)-4,5,6-trihydroxy-3-(hydroxymethyl)-2-cyclohexen-1-yl]amino]-(alpha)-D-glucopyranosyl-1(1→4)-O-(alpha)-D-glucopyranosyl-(1→4)-D-glucose.

Its empirical formula is C<sub>25</sub>H<sub>43</sub>NO<sub>18</sub>, and its chemical structure is as follows (Figure 6).<sup>3</sup> Myglitol is a monosaccharide and is chemically known as N-hydroxyethyldeoxynojirimycin. The chemical structure is as follows (Figure 7).<sup>8</sup>

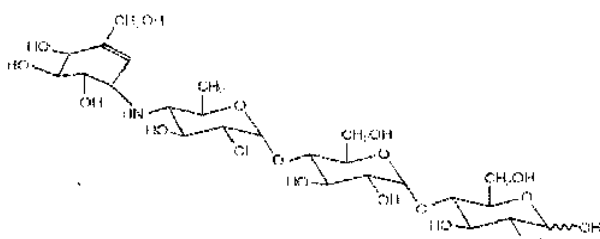


Figure 6. Chemical Structure of Acarbose

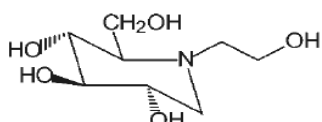


Figure 7. Chemical Structure of Mygylitol

The compounds which is contained in young and old leaf extracts of *mahkota dewa* are predicted to be carbohydrate compound that has Acarbose or Mygylitol analog structure.

## Conclusion

The young and old leaf extracts of *mahkota dewa* have inhibition activity on alpha-glucosidase; therefore, they can be used to decrease the postprandial hyperglycemia on DM patients. Ethyl acetate fraction extract from the old and young leaves has the highest inhibition activity. Methanol extract and boiled water extract from the old leaves have higher inhibition activity than the young leaves. *n*-Butanol fraction extract of the young leaves is higher than that of the old leaves.

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