

ANTIDIABETIC ACTIVITY OF MAHKOTA DEWA [*PHALERIA MACROCARPA* (SCHEFF.) BOERL.] STEM EXTRACTS AS AN INHIBITOR OF ALPHA-GLUCOSIDASE

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Abstract

Alpha-glucosidase (EC 3.2.1.20) was carbohydrase that catalyzes the liberation of α -glucose from the nonreducing end of the diet carbohydrate. In diabetic patients, inhibition of these enzymes cause restrain of glucose absorption and decrease the postprandial hyperglycemia. The purpose of this research was to study the antidiabetic activity of mahkota dewa [*Phaleria macrocarpa* (Scheff.) Boerl.] stem extracts by inhibition assay 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 assay. The alpha-glucosidase inhibition assay was performed by using alpha-glucosidase enzyme and *p*-nitrophenyl α -D-glucopyranoside as a substrate. The phytochemistry screening showed that Mahkota Dewa stem extracts contained phenolics, thanins, flavonoids, alkaloids and carbohydrates compounds. The alpha-glucosidase inhibition assay showed that at 50 ppm, inhibition activity from the ethyl acetate fraction extract of young stem (40.86%) was higher than old stem (35.22%). The inhibition activity from the *n*-butanol fraction extract of young stem (34.74%) was higher than old stem (2.57%), but for the boiled water extracts, inhibition activity from old stem (18.07%) was higher than young stem (11.38%). The methanol extract and the water fraction extract didn't have inhibition activity.

INTRODUCTION

Diabetes Mellitus (DM) is a group of symptoms that appears on someone showed by the blood glucose level that exceed the normal value (hyperglycemia) caused by insufficient insulin in the body (absolute as well as relative) (Dalimartha

2003). The occurrence phenomenon of Diabetes Mellitus disease increases from year to year. According to WHO survey, Indonesia places the fourth position in the world largest number of DM patients after India, China and The United States of America. With the prevalence of 8.6% from the total population, number of Indonesian DM patients in 1995 reached up to 4.5 million

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people, in 2001 reached 5.6 million people and in 2025 it is predicted will reach 12.4 million people (Depkes RI 2005).

DM medication can be performed with insulin injection, modern medicine such as oral antidiabetic which consist of sulfonilurea, biguanid, thiazolidinedion, and alpha-glucosidase inhibitor (Dalimartha 2003). Alpha-glucosidase inhibitor is used to medicate DM Tipe 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 hydrolyzed dietary carbohydrates to glucose. In diabetic patients, inhibition of these enzymes cause inhibition of glucose absorption and decrease the post prandial hyperglycemia. (Slagle 2002; Bayer 2004).

Mahkota dewa [*Phaleria macrocarpa* (Scheff.) Boerl.] originated from Papua (Indonesia) has been used empirically to medicate DM and other disease such as cancer, lever, rheumatic, gout, kidney, heart disease, hipertension, eczema, acne, and injury caused by insect bites (Lisdawati 2002). The extence of scientific evidence concering the hypoglycemic effect of this plant with alpha-glucosidase inhibition assay is very important in order to make this Indonesian plant became a standard herb and as a phytopharmaca supply which is medically accountable.

The purpose of this research are:

1. To perform hyperglycemic activity test from the young and old stems of mahkota dewa with alpha-glucosidase enzyme inhibition test.
2. To compare alpha-glucosidase enzyme inhibition effectivity from various young and old stem extracts of mahkota dewa.

METHODOLOGY

The research design was Complete Random Design (CRD) with four doses treatment i.e., 6.25 ppm, 12.5 ppm, 25 ppm and 50 ppm. Each treatment was replicated three times replication. The experiment unit condition was assumed to be homogen.

This research was performed in three steps. The first step was fractionated and extracted sample from the young and old stems 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 assay on various stem extracts.

Fractionation and Extraction

Fractionation and extractions were as follows: 200 gram mahkota dewa old stem powder is put in 1.5 litre methanol solvent for 4 days at 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 stem is obtained with same way as old stem methanol extract. Further, methanol extract is fractionated with the mixture of water and

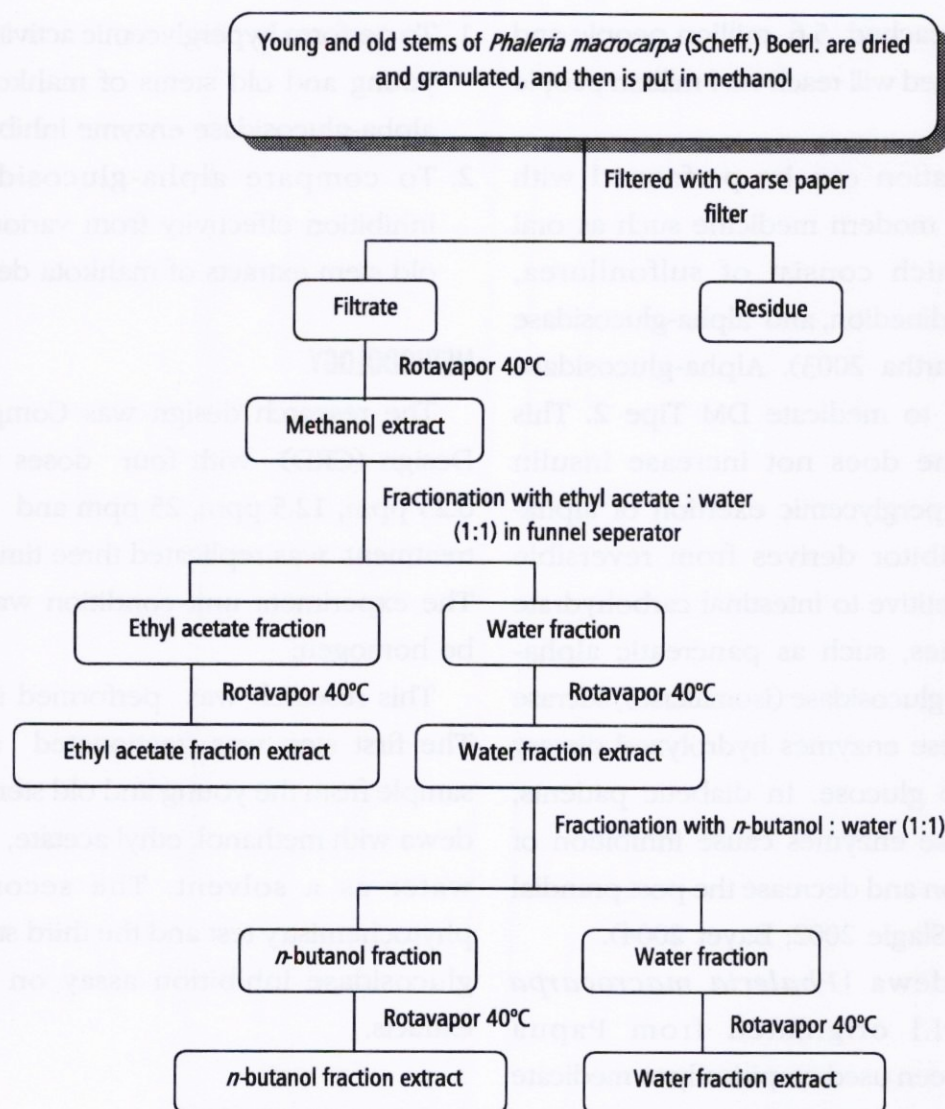


Figure 1. Fractionation and extraction chart of the mahkota dewa stem

ethyl acetate solvent (1:1) to obtain ethyl acetate fraction and water fraction. Water fraction is refractionated by *n*-butanol and obtained *n*-butanol fraction and water fraction. Each fraction is concentrated by rotary evaporator at 40°C until ethyl acetate fraction extract, *n*-butanol fraction extract, and water fraction extract are obtained.

Fractionation and extraction of samples could be seen in Figure 1. Water extract was also obtained by boiling the stem of mahkota dewa.

Phytochemistry Test

Phytochemistry test were consist 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_2SO_4 , and Biuret test with NaOH dan $CuSO_4$.

Alpha-Glukosidase Inhibition Assay (Lee and Lee, 2001; Prashanth *et al.*, 2001)

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. 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 mL 20 mM *p*-nitrofenil α -D-glukopiranoside as a substrate, 490 ml 100 mM phosphat buffer (pH 7,0) and 10 mL solution sample in DMSO. After the mixture incubated at 37°C for 5 minutes, 250 mL enzyme solution is added and incubated for 15 minutes. Enzymatic reaction is stopped by adding 1000 mL 200 mM sodium carbonate and the the absorbance of *p*-nitro phenol 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.

Tabel 1. Enzyme reaction system for one sample with 2 mL total volume

	Blank μ L	control μ L	So μ L	S1 μ L
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_2CO_3	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 stems with 1%, 0.5%, 0.25% and 0.125% concentration variation and DMSO as a solvent. For each extract we perform alpha-glucosidase inhibition test with 3 times repetition (triplo). The inhibition percentage could be calculated with equation:

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

S = sample absorbance (S1-So),

S1 = sample absorbance with enzyme addition,

So = sample absorbance without enzyme addition,

C = absorbance control (DMSO) without sample (control-blank)

RESULT AND DISCUSSION

Phytochemistry Test

In phytochemistry test to methanol extract, ethyl acetate fraction extract, *n*-butanol fraction extract, water fraction extract and boiled water extract from young and old stems of mahkota dewa can be identified the compound class of phenolic, thanin, flavonoid, alkaloid and carbohydrate.

Alpha-Glukosidase Inhibition Assay

Inhibition assay on alpha-glukosidase enzyme is performed to determined the antihyperglycemic activity of all extracts. In this experiment, alpha-glucosidase enzyme will hydrolyse *p*-nitrophenyl α -D-glucopyranoside become *p*-nitrophenol with yellow color and glucose with the following reaction:

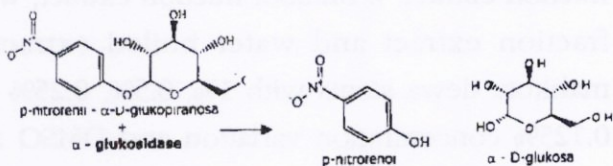


Figure 2. The enzymatic reaction of α -glucosidase *p*-nitrophenyl α -D-glucopyranoside with α -glucosidase enzyme

Enzyme activity is measured based on *p*-nitrophenyl absorbance result in yellow color. The extence of stem extracts that function as alpha-glucosidase inhibitor then *p*-nitrophenyl obtained will lessen as shown by the lessening yellow color intensity (Basuki *et al* 2002).

Figure 3 illustrates inhibition percentage to alpha-glucosidase enzyme of old stem extracts at concentration of 6.25 ppm, 12.5 ppm, 25 ppm and 50 ppm.

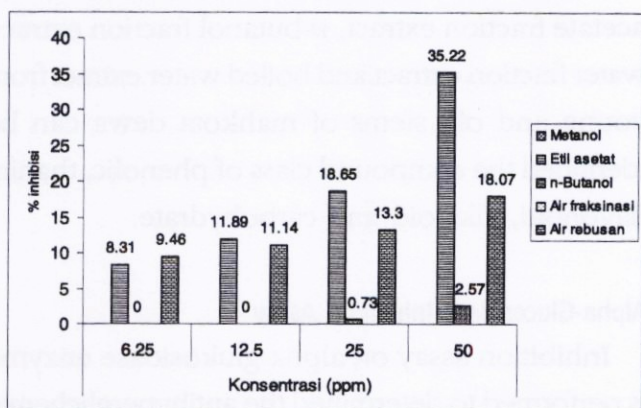


Figure 3. Inhibition percentage to alpha-glucosidase enzyme of methanol extract, ethyl acetate fraction extract, *n*-butanol fraction extract, water fraction extract and boiled water extract of mahkota dewa old stem.

Ethyl acetate fraction extract had the highest inhibition activity followed by boiled water extract in almost all the test concentrations. *n*-Butanol fraction extract had very low inhibition activity, and only had inhibition activity at 50 ppm and

25 ppm. Methanol extract and water fraction extract didn't have inhibition activity in almost all the test concentrations.

Figure 4 illustrates inhibition percentage to alpha-glucosidase enzyme of young stem extracts at concentration of 6.25 ppm, 12.5 ppm, 25 ppm and 50 ppm.

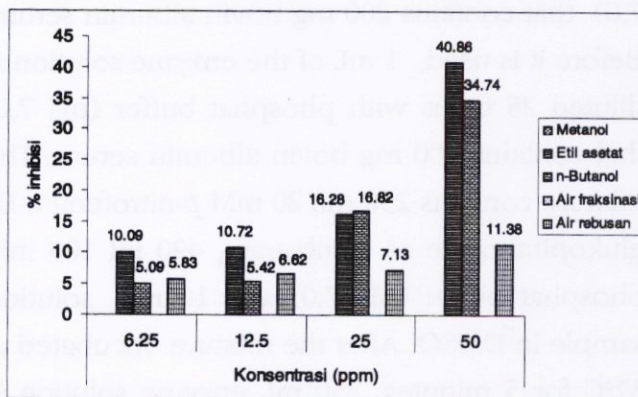


Figure 4. Inhibition percentage to alpha-glucosidase enzyme of methanol extract, ethyl acetate fraction extract, *n*-butanol fraction extract, water fraction extract and boiled water extract of mahkota dewa young stem.

Ethyl acetate fraction extract had the highest inhibition activity followed by *n*-butanol fraction extract and water boiled extract on almost all of the concentration tested. Methanol extract and water fraction extract didn't have inhibition activity on almost all the concentration tested.

The existance of inhibition activity to alpha-glucosidase enzyme from various extracts of the mahkota dewa stems possibility due to the carbohydrate compound which thought to be a competitive inhibitor of alpha-glucosidase enzyme. It is consistent with the substrate of alpha-glucosidase enzyme, food carbohydrate such as starch and glycogen. In addition, these allegations

were also based on the type of alpha-glucosidase inhibition drug such as Acarbose and Myglitol which is a carbohydrate compound. Acarbose is an oligosaccharide which is obtained from the microorganism fermentation of *Actinoplanes utabensis* and the chemical name is 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. The empirical formula is $C_{25}H_{43}NO_{18}$ and the chemical structure in Figure 5 (Slagle 2002; Bayer 2004).

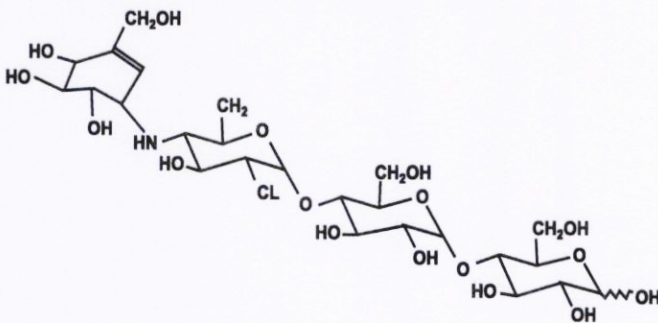


Figure 5. Chemical structure of Acarbose

Myglitol is a monosaccharide which chemical name is N-hydroxyethyldeoxynojirimycin and the chemical structure in Figure 6 (PharmaChem. 2003).

The compound which is in the stem extract of mahkota dewa is suspected a carbohydrate compound. The structure of the compound is suspected analogous with Acarbose or Myglitol.

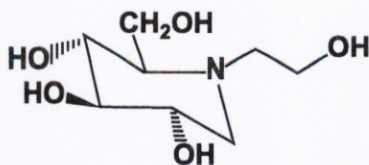


Figure 6. Chemical structure of Myglitol

CONCLUSION

Ethyl acetate fraction extracts from the young and old stems of mahkota dewa have the highest inhibition activity. Water boiled extract of the old stem has higher inhibition activity than the young stem. The inhibition activity from *n*-butanol fraction extract of the young stem is much higher than the old stem. Methanol extract and water fraction extract didn't have inhibition activity.

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