

Ethanol extract of Mahkota Dewa (*Phaleria macrocarpa* (Scheff.) Boerl.) fruit with *in-vitro* antidiabetic activities

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Abstract

Mahkota dewa (*Phaleria macrocarpa*) fruit is popular in Indonesia used as an alternative or traditional medicine especially for antidiabetic. However, its mechanism and compound as antidiabetic has not been further studied yet. Therefore, the objective of this research is to elucidate its mechanism of action as insulin secretagog in clonal glucose responsive insulin secreting cells BRIN-BD11 and α -glucosidase inhibitor *in-vitro*. The extract was further analyzed to determine its active compound. Mahkota dewa's fruits were extracted by maceration method with ethanol 30% as solvent. The ethanol extract contains phytochemical alkaloid, flavonoid, tannin, and steroid. Insulin secretory responds after one hour incubation in 16.7 mM glucose media with concentration of 1.12, 2.25, and 4.50 mg/ml mahkota dewa fruit extract showed 1.5, 1.3, and 2.0 folds increased compared to control. However, almost the same respond compared to glibenclamide, which is an insulin secretagog drug. In-vitro assay for α -glucosidase inhibitory activity was measured based on the formation of *p*-nitrophenol (yellow solution) through hydrolysis of substrate *p*-nitrophenyl α -D-glucopyranoside. The concentration of the extract were 1%, 1.5%, and 2% resulted inhibition level respectively 26.40%, 29.22%, and 24.51%, which was very low compared to acarbose (99%), an α -glucosidase inhibitor. The extract was further analyzed with flash chromatography, thin layer chromatography, and reconfirmed with phytochemical assay which contains flavonoid compound.

Keywords: α -glucosidase inhibitor, flavonoid, insulin secretagog

Introduction

Diabetes mellitus is a condition or disease caused by deficiency in insulin. The characteristic of this disease is high blood glucose concentration or hyperglycemia. World Health Organization (WHO) reported that in 2025 there will be an estimation of 300 millions people in the world suffered diabetes. In 2005, Indonesia was rank 4th in the world and continues to increase to 12.4 millions people in the year 2025 (Department of Health 2005).

It is necessary to find new approaches for cure and prevention of this disease that is affordable for the people. Several mechanisms for oral antidiabetic are insulin secretagog that stimulate pancreas to secrete insulin and α -glucosidase inhibitors that block the enzyme α -glucosidase in the brush borders of the small intestines, which delays absorption of carbohydrates (Fonseca & Kulkarni 2008; Asano 2003). Efforts in finding cure with using herbal plants had been pursued for many years. However, WHO had recommended that traditional plant therapy for diabetes need further evaluation (WHO 1980).

Mahkota dewa (*Phaleria macrocarpa*) is a member of the Thymelaeaceae family. It has been reported that this plant has several medicinal properties including the ability to lower blood

pressure, to cure hepatitis, to increase stamina, to treat cancer and to lower blood glucose (Harmanto 2003; Winarto 2003).

Empirically, the fruit of mahkota dewa (*Phaleria macrocarpa*) believed to reduce blood glucose and had been shown in a preliminary study using diabetic induced-streptozotocin rats. The water extracted fruit of mahkota dewa reduced 46% of the blood glucose compared to its control group with dosage of 32.1 mg/kg body weight (Shalahuddin *et al.* 2005). However, its mechanism and the phytochemical compound as antidiabetic still unknown. Therefore, in this study, the goal is to understand the phytochemical compound and the *in vitro* mechanism of the ethanol extract of mahkota dewa fruits as insulin secretagog in pancreatic cell culture BRIN-BD11 and as α -glucosidase inhibitor.

Materials and Methods

Reagents

α -Glucosidase of *Bacillus stearothermophilus* (Sigma-Aldrich, USA), *p*-nitrophenyl- α -D-glucopyranoside (Sigma-Aldrich USA), bovine serum albumin (Merck-Darmstadt), Na₂CO₃, acarbose (Bayer, Indonesia), rat insulin ELISA (Mercodia,

Sweden), RPMI medium, Krebs Ringer's Buffer and Hepes.

Preparation of ethanol extract

Ripe fruit of mahkota dewa (*Macrocarpa phaleria*) that has dark pink with slight green streaks from the product of Faculty of Forestry Bogor Agricultural University Indonesia were sliced thinly, dried in oven (50°C) for 30 hours and made into powder. The powder was extracted with ethanol 30% with maceration techniques for three days then, dried using rotary evaporator.

Qualitative identification of phytochemical compound

Phytochemical qualitative assay of the ethanol extract of mahkota dewa fruit was performed according to the method described by Harborne (1987) for flavonoid, alkaloid, saponin, tannin, triterpenoid, and steroid compound.

α -Glucosidase inhibitor activity in vitro

α -Glucosidase inhibitor activity was determined using modified method by Watanabe *et al.* 1997. Extracted sample was dissolved in dimethylsulfoxide (DMSO) at a concentration of 1%, 1.5% and 2%. Enzyme 1.0 mg α -glucosidase dissolved in 100 mM phosphate buffer (pH 7) containing 200 mg bovine serum albumin. Mixed solution consisted of 250 μ l *p*-nitrophenyl- α -D-glucopyranoside (*p*-NPG) 200 mM as substrate, 490 μ l phosphate buffer (pH 7) 100 mM, and 10 μ l sample in DMSO 1%. This mixture was incubated at 37°C for 5 minutes, added 250 μ l enzyme solution then additional 15 minutes incubation. Reaction was stopped with the addition of 1000 μ l sodium carbonate (Na₂CO₃) 200 mM. The activity was measured with spectrophotometer at λ 400 nm. Positive control was *acarbose* tablet in phosphate buffer (pH 7) and HCl 2N with standard concentration of 1%.

Insulin secretagog in vitro

Cell culture BRIN-BD11, a glucose-responsive clonal insulin-secreting cell line, produced by electro fusion of immortal RINm5F cell with New England Deaconess Hospital rat pancreatic B-cell, was used to evaluate insulin secretion (McClenaghan *et al.* 1996, Gray & Flatt 1997). This cell culture used for screening of antidiabetic plant materials and characterization of novel insulin-releasing natural products has been described elsewhere (Gray & Flatt 1997a,b 1998). Cells were seeded at a concentration of 2x10⁶ cells/well in 24-well plates (Falcon, NJ, USA) cultured in RPMI-1640 containing 11.1 mM glucose, 10% fetal calf serum and antibiotics (50.000 IU/l penicillin-streptomycin) to allow attachment overnight prior to acute tests. Cells were washed three times with Krebs-Ringer bicarbonate buffer (KRB; 115 mM NaCl, 4.7 mM KCl, 1.3 mM CaCl₂, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, 24 mM NaHCO₃, 10 mM

Hepes-free acid, 1 g/l bovine serum albumin, 1,1 mM glucose; pH 7.4) and preincubated for 40 min at 37°C. Cells were then incubated for 20 min with 1 ml KRB at 1.1 mM glucose or 60 min in KRB 16.7 mM in the absence and presence of plant extract. Following incubation, aliquots were removed from each well and stored at 20°C for insulin assay (Flatt & Bailey 1981).

Flash chromatography of the extract

Fractionation of the crude extracts using flash chromatography with the velocity of 10 mL/min. The eluent used was mixture of butanol:acetate acid:water with ratio 60:15:25. Those fractions were further collected into one fraction based on the R_f value with the thin layer chromatography then identified by UV spectrophotometer.

Results and Discussion

Phytochemical compound

The phytochemical compound of the ethanol extract of mahkota dewa fruit consist of alkaloid, flavonoid, tannin and steroid. This finding was different compared to Wulandari (2005) and Shalahuddin (2005). They reported that it also contain saponin. Phytochemical compound is a secondary metabolite which is affected by geographical and micronutrient of the soil (Kardono 2003)

α -Glucosidase inhibitor activity in vitro

The inhibitory activity of the ethanol extract of mahkota dewa fruits is shown in Figure 1. The inhibitory activity in the concentration of 1%, 1.5%, and 2% were 26.48%, 29.22%, dan 24.51%, respectively. The positive control, acarbose 1% had 99.34% inhibitory activity against α -glucosidase. However, the inhibitory activity of the methanol extract of mahkota dewa fruits with concentration of 1% were 40.9% which is higher compared to the ethanol extract (Rohimah 2008). Mai and Chuyen (2007) reported that *Sophora japonica*, *Nelumbo nucifera*, *Psidium guajava*, *Camellia sinensis* and *Cleistocalyx operculatus* with concentration 20 mg/ml had inhibitory activity respectively 47.5%, 51.4%, 60.8%, 65.4%, and 68.2%.

α -Glucosidase is an important enzyme for carbohydrate in the small intestine which controls the post-prandial blood glucose concentration (Mooradian & Thurman 1999; Fonseca & Kulkarni 2008). Synthetic α -glucosidase inhibitory agent, acarbose has more side effects such as flatulence and diarrhea (Mooradian & Thurman 1999), therefore, natural product is hoped to have less side effect. The lower inhibitory effect of mahkota dewa fruit extract is potential to be used as antidiabetic but need further evidence through *in vivo* study.

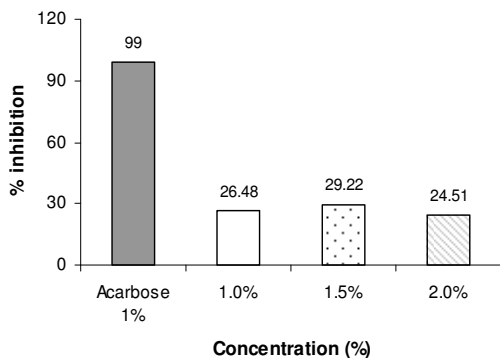


Figure 1 α -Glucosidase inhibitory activities of ethanol extract of mahkota dewa fruit with concentration of 1.0%, 1.5%, and 2%, with positive control, acarbose 1%

Insulin secretagog in vitro

Ethanol extract of mahkota dewa fruits with varied concentration showed stimulatory effect on insulin secretagog in cell culture of BRIN-BD11 with 16.7 mM glucose with 60 min incubation compared to the 20 min incubation (Table 1).

Table 1 Effect of ethanol extract of mahkota dewa fruits on insulin secretion at 1.1mM glucose (A), 16.7 mM glucose at 20 min (B) and 16.7 mM glucose at 60 min (C).

Extract concentration (mg/ml)	Insulin secretion ($\mu\text{g/l}/3 \times 10^5$ cells)		
	A	B	C
0	0.262	0.180	0.253
1.125	0.210	0.200	0.380
2.250	0.237	0.276	0.332
4.500	0.250	0.290	0.506

The insulin secretion for concentration of 1.125mg/ml, 2.250 mg/ml and 4.500 mg/ml was 1.5 fold, 1.3 fold, and 2 folds respectively compared to control. The increased response is only for the 60 min incubation in the present of higher glucose concentration (16.7 mM) which represent the second phase of biphasic insulin secretion (Mears & Atwater 2000). However, the 20 min incubation (as the first phase) showed no response. This result showed that mahkota dewa ethanol extract had lower insulin secretion response compared to sambiloto with 3.7 fold increased for concentration of 2.5 mg/ml (Wibudi 2006).

Flash chromatography of the extract

Fractionation of the ethanol extract of mahkota dewa fruit was processed with flash chromatography. The separation of compound with mixture of butanol:acetate acid:water in the ratio of 60:15:25 that showed optimal result. Further separation was

performed using thin layer chromatography that result four fractions. Identification of the fractions with phytochemical assays and spectrophotometer ultraviolet (wavelength 259-260) suggesting of flavonoid compound (Harborne 1987).

Conclusions

Ethanol extract of mahkota dewa fruit has *in vitro* α -glucosidase inhibitor activities and insulin secretagog. The phytochemical compound that showed activities as antidiabetic was suggested as the flavonoid compound. However it needs further exploration.

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