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Alpha-Glucosidase Inhibition and Hypoglycemic Activities of *Sweitenia mahagoni* Seed Extract

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Inhibition of α-glucosidase and hypoglycemic activity are two effects commonly used to identify bioactive compounds with potential to treat diabetes. The objectives of this study were to analyse and compare the bioactive compounds and α-glucosidase inhibitory effect of four different types of *Swietenia mahagoni* seed extract, and to analyse the hypoglycemic activity of the greatest inhibition of α-glucosidase-extract in rats. The extracts were obtained using two different solvents (aqueous and ethanol) and two different methods: maceration and reflux methods. This resulted in four types of extract varying by solvent and extraction method. Testing of these extracts for α-glucosidase inhibitory effect was carried out *in vitro* using spectrophotometer. Testing for hypoglycemic activity was carried out *in vivo* using rats. A total of 40 male *Sprague-Dawley* rats were divided into eight groups: (1) the negative control group, received an oral dose of aquadest only, (2) the positive control group, was given 90% sucrose orally without *S. mahagoni* seed extract, and five treated groups (3-7), were given 90% sucrose followed by the best extract-ethanolic *S. mahagoni* seed extract in doses of 100, 200, 300, 400, and 500 mg/kgBW, and (8) the acarbose group, was given 90% sucrose orally followed by acarbose. Glucose levels in each animal were measured at 0, 30, 60, 90, and 120 min after treatment. The results showed the greatest inhibition of α-glucosidase in ethanolic extract, using maceration methods. This ethanolic-maceration *S. mahagoni* seed extract also showed hypoglycemic effects in hyperglycemic rats at dose from 100 to 500 mg/kgBW. Ethanolic extract of *S. mahagoni* seed, using maceration method, can be proposed as potential antidiabetic agent.

Keywords: *Swietenia mahagoni*, α-glucosidase, hypoglycemia, ethanolic extract, antidiabetic

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease caused by an absolute or relative lack of insulin secretion, low insulin sensitivity, or both. Insulin is needed to uptake glucose from blood circulation into the cells. DM interferes with this process, causing impairment of carbohydrate, protein and lipid metabolism. This disease characterized by abnormally high plasma glucose levels, leading to major complications, such as neuropathy, retinopathy, and cardiovascular disease. Effective control of blood glucose levels is key for preventing or reversing diabetic complications and improving the quality of life for diabetic patients (Bell 2001).

The number of DM patients increases every year. The number of diabetics in Indonesia is expected to increase from 8.4 million people in 2000 to 21.3 million in 2030 (IDF 2013). According to the World Health Organization (WHO), Indonesia ranks 4th highest in the world in number of DM patients, after China, India, and the United States of America (USA).

DM is also a disease found in pet animals, especially dogs and cats (Rand & Marshall 2005; Wiedmeyer & DeClue 2011). The number of pets diagnosed also increases every year. Becker (2010) reported that there are more than 1.4 million diabetic dogs and cats in the USA with high of blood glucose levels (http://healthypets.mercola.com/sites/healthypets/archive). Diabetes rates in dogs increased 200% over a 30 year period (http://sciencedaily.com/releases/2011/04/1104251). This same trend holds true in Indonesia, where rates of DM in pets (dogs and cats) increase every year. Diabetes in dogs and cats is usually traced to predisposing factors such as obesity, or inflammation of the pancreas. DM may also be caused by treatment with glucocorticoids which can disturb insulin function. In pets, DM used to be seen mainly in older animals, but recently has been on the rise in relatively young animals as well. DM occurs predominantly in female dogs and male cats (Washington State University 2013: http://www.vetmed.wsu.edu/cliented/diabetes.aspx).

In order to address the problem of diabetes mellitus in both human and animals, it is important

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to search for biochemical compounds that may serve as antidiabetic treatments. Our study examines the antidiabetic potential of *Swietenia mahagoni*, a tree found almost everywhere in Indonesia. Studies report that Indian *Swietenia mahagoni* has antioxidative and antidiabetic activities in streptozotocin-induced rats (Panda *et al*. 2010). De *et al*. (2010) also suggested that Indian *Swietenia mahagoni* is a good candidate for antidiabetic medicine. However, no antidiabetic product has yet been produces using *Swietenia mahagoni* seed. The *Swietenia mahagoni* seeds used in this study were obtained from Leuwiliang Bogor, Indonesia. Little is known about the potency of *Swietenia mahagoni,* but we know that bioactive compounds found in plants may depend on the location where they grow. This study of the antidiabetic properties of *Swietenia mahagoni* will also provide information specific to plants grown in Leuwiliang Bogor, Indonesia. This study is a preliminary study to explore the potential for producing an antidiabetic product using *Swietenia mahagoni* seed. The objective of this study was to analyse the inhibitory activity of *Swietenia mahagoni* seed extracts to *in vitro* alpha-glucosidase and the *in vivo* hypoglycemic activity of the extract in sucrose-induced hyperglycemic rats.

MATERIALS AND METHODS

Materials. *Swietenia mahagoni* seeds were harvested from the wild in Leuwiliang, Bogor, Indonesia. Sampling was done using WHO procedure (2000). The seeds were first dried at 50 °C, then were ground and filtered with 40 mm mesh. A total of 40 male *Sprague-Dawley* rats were used in this study. The rats were obtained from Animal Laboratory Unit, Faculty of Veterinary Medicine, Bogor Agricultural University, Indonesia.

***Swietenia mahagoni* Seeds Extraction.** Extraction was carried out using two methods, maceration and reflux. We also used two solvents, aquadest and ethanol. This resulted in a total of four extracts: (i) aquaeus-maceration extract, (ii) ethanol-maceration extract, (iii) aquaeus-reflux extract, (iv) ethanol-reflux extract (Figure 1). The maceration method was as follows: *Swietenia mahagoni* seeds were dried and ground into powder, which was mixed with solvent (1:5) for 24 h, followed by filtration. The maceration was repeated three times with the same solvent. All of the filtrate was then evaporated using vacuum evaporator. Maceration using aquadest was carried out using aquadest at 80-90 °C. Reflux extraction was carried out over 6 h, using a 1:5 proportion of sample to solvent, then left overnight.

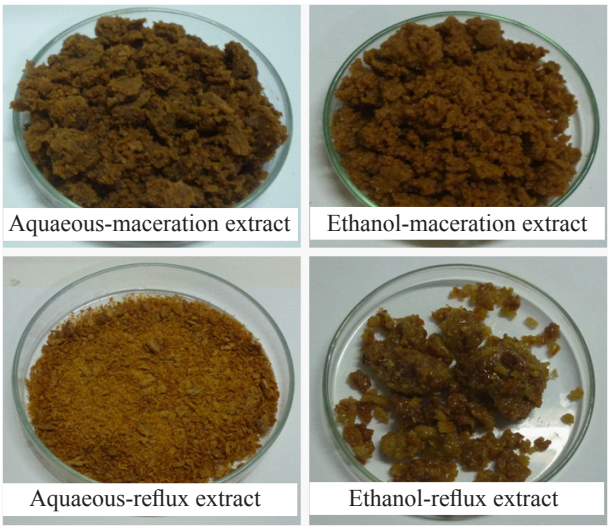

Figure 1. *Swietenia mahagoni* seed extracts.

The resulting filtrate was then filtered, and evaporated using vacuum evaporator. All of the resulting four extracts were then analyzed for phytochemical compounds, including alkaloids, phenols, flavonoids, etc. (Harborne 1987).

***In vitro*–Inhibitory Activity of Extracts on α-Glucosidase.** We created an enzyme solution consisting of 1.0 mg α-glucosidase in 100 mL phosphate buffer (pH 7.0) containing 200 mg bovine serum albumin (BSA). One mL of this enzyme solution was then diluted 25 times with phosphate buffer. The reaction solution consisted of 250 µL 20 mM p-nitrophenyl α-D-glucopiranose (as a subtrate), 490 µL phosphate buffer, 10 µL extract, and 10 µL DMSO. The reaction solution was then incubated in water bath at 37 °C for 5 min, then amended with 250 µL enzyme solution, followed by incubation again for 15 min. The reaction was then stopped via the addition of 1000 µL 200 mM sodium carbonate. The absorbancy of the reaction result was measured at λ = 410 nm. Percent inhibition was then calculated as follows: [(C-S)/C] x 100%, S = sample absorbancy (S1-S0), S1 = sample absorbancy with enzyme, S0 = sample absorbancy without enzyme, and C = control absorbancy (DMSO) without sample.

***In vivo*-Hypoglycemic Activity of Extract on Sucrose-Induced Hyperglycemic Rats.** Rat care and experimental procedures in this study were in accordance with Ethical Approval Letter No. 14-2013 IPB from Animal Care and Use Committe, Bogor Agricultural University. A total of 40 male Sprague-Dawley rats were used for this study. They were divided into eight groups: a negative control group, a positive control group, and five groups that were treated with the extract, that showed the highest inhibitory activity on alpha-glucosidase (that is ethanol-maceration extract of *Swietenia mahagoni*

seed) at dose 100, 200, 300, 400, and 500 mg/kgBW, and an acarbose (4.5 mg/kgBW) treated group. All the rats were made to fast for 10 h, then blood glucose level were measured to serve as the 0 minute baseline level. The rats were then given an orally administrated dose of aquadest (negative control and positive control groups), ethanol maceration extract of *Swietenia mahagoni* seed (extract treated groups) at dose 100, 200, 300, 400, and 500 mg/kgBW, or acarbose (acarbose treated group). After 10 min, all of rats were given an orally administrated 1 mL 90% sucrose, except for the negative control group. The negative control group was given an orally administrated aquadest, as placebo. Blood glucose levels for the rats were then measured at 30, 60, 90, and 120 min after sucrose treatment. Blood glucose was measured using a Gluko Dr and strip kit.

RESULTS

Phytochemical Characteristic of *Swietenia mahagoni* Seed Extract. *Swietenia mahagoni* seed extracts were showed in Figure 1. There are four extracts in this study, aquaeous-maceration, ethanol-maceration, aquaeous-reflux, and ethanol-reflux extract. The characterization all of extracts were showed in Table 1, including water and ash content, as well as the extract yield, and the levels of phytochemical coumponds such as alkaloids, saponins, steroids, tannins, triterpenoids, hydroquinones, and flavonoids.

The extract yield was highest using the maceration method and ethanol as a solvent (41.67%). The yield of aquaeous-maceration, aquaeous-reflux, and ethanol-reflux extract were 28.87, 14.95, and 21.61%, respectively. All four types of *Swietenia mahagoni* seed extracts showed a different composition of alkaloids, saponins, steroids, tannins, triterpenoids, and hydroquinones in variation. The total flavonoid content (in % with quercetin) was highest in the ethanol-maceration extract (0.706%). The total flavonoid content (in % with quercetin) of aquaeous-maceration, aquaeous-reflux, and ethanol-reflux extract were about 0.2% each (Table 1).

Alpha-Glucosidase Inhibitory Activity of *Swietenia mahagoni* Extract. The inhibitory effect of *Swietenia mahagoni* extracts to α-glucosidase is shown in Table 2. The ethanol-maceration extract showed the highest inhibitory activity, measured 18.647 percent inhibition at 100 ppm. The results for the other extracts were lower; ethanol-reflux extract (14.313), followed by aquaeos -reflux extract (5.309), and aquaeous-maceration extract (4.376). Based on these results and on the flavonoid content found in the extract (Table 1), the ethanol-maceration extract showed the greatest promise as an antidiabetic agent. Therefore, the hypoglycemic effect analysis of *Swietenia mahagoni* seed extract was carried out using only the ethanol-maceration extract.

Hypoglycemic Activity of *Swietenia mahagoni* Extract in Sucrose-Induced Hyperglycemic Rats. The results of hypoglycemic effect analysis of ethanol-maceration extract in *Sprague-Dawley* rats at dose 100, 200, 300, 400, and 500 mg/kgBW

Table 2. α-glukosidase inhibitory activity of *Swietenia mahagoni* seed extract (at 100 ppm extract)

Swietenia mahagoni seed extract	% Inhibition
Aquaeos maceration	4.376 ± 0.192a
Ethanol maceration	18.647 ± 3.864b
Aquaeos reflux	5.309 ± 0.514a
Ethanol reflux	14.313 ± 3.522b

Table 1. The characteristic of *Swietenia mahagoni* seed extract

Parameter		Aqueos maceration extract	Ethanol maceration extract	Aqueos reflux extract	Ethanol reflux extract
Water content		13.58	2.22	9.57	2.88
Ash content		5.42	2.48	3.26	2.23
Acid-insoluble ash content		0.35	0.12	0.31	0.21
Yield (%)		28.87	41.67	14.95	21.61
Organoleptic:	Form	powder	powder	powder	powder
	Smell	specific	specific	specific	specific
	Colour	brown	brown	brown	brown
Phytochemistry:					
Alkaloid:	Wegner	negative	positive	positive	negative
	Meyer	positive	positive	negative	negative
	Dragendorf	negative	negative	negative	negative
Steroid		positive	negative	positive	negative
Tannin		negative	negative	negative	negative
Saponin		positive	positive	positive	positive
Triterpenoid		positive	positive	negative	positive
Hidroquinone		negative	positive	negative	negative
Total flavonoid : quersetin % (w/w)		0.267	0.706	0.297	0.268

are shown in Figure 2. The negative control group showed normal blood glucose levels at 0, 30, 60, 90, and 120 min. The blood glucose levels of the positive control group which received 90% sucrose solution without *S. mahogoni* seed extract increased to 150 mg/dL at 30 min after sucrose treatment. The blood glucose levels peaked (170 mg/dL) at 60 min, and then decreased at 90 min, reaching normal levels 120 min after sucrose solution treatment. The positive control group were hyperglycemic at testing intervals 30 and 60 min after receiving sucrose solution. The rats in the groups that were treated with sucrose solution and *Swietenia mahagoni* seed extract at all dose from 100 through 500 mg/kgBW showed slightly increased but normal blood glucose levels at the 30 and 60 min test time, with decreased (but normal) levels at min 90 and 120. All of the groups treated with *Swietenia mahagoni* seed extract exhibited blood glucose levels within normal range during the entire 120 min (Figure 2).

DISCUSSION

Swietenia mahagoni seed extract displayed the presence of bioactive compounds, such as alkaloids, steroids, tannins, saponins, triterpenoids, hidroquinons, and flavonoids. All of these bioactive compounds possess certain physiological functions. To determine if any of the functions might be antidiabetic, we tested for both α-glucosidase inhibition and hypoglycemic effects of *Swietenia mahagoni* seed extract. An extract derived using ethanol solvent and the maceration method showed the best results, compared to extracts using other methods and solvents, in term of highest yield and flavonoid contents. *In vitro* analysis indicated that the ethanolic-maceration extract also showed the greatest α-glucosidase inhibition activity than the other extracts. This suggested that levels of α-glucosidase inhibitors may be correlated with alkaloid, triterpenoid, and flavonoid levels in the *Swietenia mahagoni* seed extract (Table 1). Benala *et al*. (2010) reported that naturally abundant antioxidant compounds have received considerable attention as potential α-glucosidase inhibitors. Alkaloids (Patel & Mishra 2012), triterpenes (Lai *et al*. 2012), and flavonoids (Wang *et al*. 2010) have all been reported to exhibit α-glucosidase inhibitor properties.

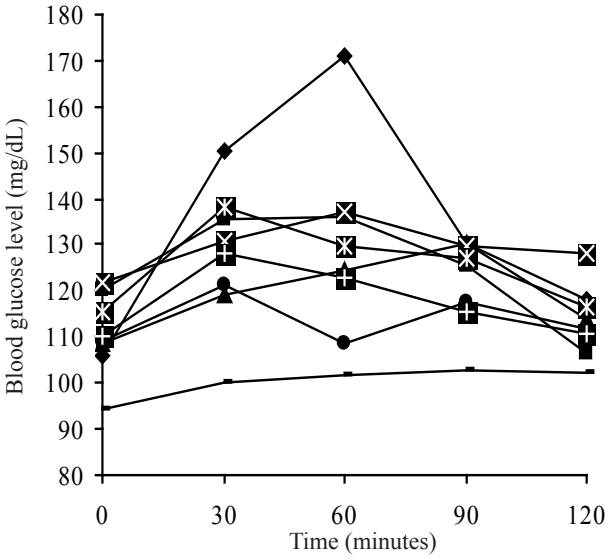

Figure 2. Blood glucose level of rats. The positive control group (hyperglycemic) showed the highest blood glucose level, especially at 30 and 60 min after oral treatment with sucrose. Rats treated with ethanol *Swietenia mahagoni* seed extract (100, 200, 300, 400, and 500 mg/kgBW) showed lower blood glucose levels than rats in the positive control group. — Negative control, ◆ Positive control, ■ 100 mg/kgBW, ▲ 200 mg/kgBW, ✕ 300 mg/kgBW, ✕ 400 mg/kgBW, ● 500 mg/kgBW, ✚ Glucobay.

In vivo analysis showed that *Swietenia mahagoni* seed extract, especially ethanolic extract, has a hypoglycemic effect in sucrose-induced hyperglycemic rats. This is indicated by the fact that blood glucose measurement in all of the rats treated with *Swietenia mahagoni* seed extract never reached hyperglycemic levels during the 120 min observation period for 120 min. Normal blood glucose levels for rats range between 90-142 mg/dL (Kim *et al*. 2006), or 99-127 mg/dL (Gulfraz *et al*. 2007). The positive control group, which received the 90% sucrose solution without *Swietenia mahogoni* seed extract treatment, exhibited hyperglycemic blood glucose levels at 30th and 60th min after sucrose treatment. This hyperglycemic condition indicates increased absorption of glucose in the small intestine as a result of sucrose hydrolysis. In contrast, rats treated with sucrose and with *Swietenia mahagoni* seed extract were able to suppress this process and maintain normal blood glucose measurement. These results suggest that *Swietenia mahagoni* seed ethanolic-maceration extract, works in a similar way as acarbose treatment to lower blood glucose level. The *Swietenia mahagoni* seed ethanolic-maceration extract may inhibit the activity of α-glucosidase in the small intestine as showed at *in vitro* analysis, delaying sucrose digestion, and thus reducing the rate of glucose absorption. Consequently, normal blood glucose level can be maintained despite treatment with a dose of sucrose that would ordinarily cause a hyperglycemic condition.

Diabetes is a common metabolic disease characterized by abnormall high plasma glucose levels (hyperglycemia), leading to major complications, such as diabetic neuropathy, retinopathy, and cardiovascular diseases (Kumar *et al*. 2011). Hyperglycemia is a metabolic disorder primarily characterized by a disorder of the β-cells, relative insulin deficiency, and an abnormal rise in blood glucose, right after a meal (Kwon *et al*. 2007). One effective strategy to manage diabetes mellitus, is to decrease postpandrial hyperglycemia by retarding the absorbtion of glucose. This is achieved by inhibition of carbohydrate hydrolyzing enzymes, such as α-glucosidase and α-amylase, in the digestive organs (Kumar *et al*. 2011). α-Glucosidase is the key enzyme catalyzing the final step in the digestive process of carbohydrates. Hence, α-glucosidase inhibitors can retard the liberation of d-glucose from dietary complex carbohydrates, and so delay glucose absorption in the small intestine, resulting in reduced postpandrial plasma glucose levels and suppression of postpandrial hyperglycemia (PPHG). PPHG is a major risk factor for diabetic vascular complications leading to disability and mortality in diabetics (Shihabudeen *et al*. 2011). As reported by Bischoff (1995) α-glucosidase inhibitors via competitive and reversible inhibition of intestinal α-glucosidases, delay carbohydrate digestion, prolongs overall carbohydrate digestion time, and thus reduces the rate of glucose absorption. Casirola and Ferraris (2006) also reported that one potential mechanism by which α-glucosidase inhibitors blunt postpandrial glycemic spikes and lower fasting blood glucose concentration is by preventing carbohydrate-induced increases in intestinal sugar transport. Sustained reduction in hyperglycemia will decrease the risk of developing microvascular complications and most likely will reduce the risk associated with microvascular complications (Tas *et al*. 2005). In animal subjects, some researches show that a low-carbohydrate diet supplemented with acarbose-α-glucosidase inhibitor was an effective means of decreasing exogenous insulin dependence and improving glycemic control in cats diabetes mellitus (Mazzaferro *et al*. 2003).

This study, both *in vitro* and *in vivo,* concluded that *Swietenia mahagoni* seed ethanolic-maceration extract has α-glucosidase inhibitory and hypoglycemic effects. The results suggest that the extract should be proposed as a potential antidiabetic treatment that lowers blood glucose, via inhibition of α-glucosidase. Raptis and Dimitriadis (2001) described various mechanisms by which oral hypoglycemic agents may work: as α-glucosidase inhibitors, insulin secretagogues, and insulin sensitizers. These and other potential antidiabetic mechanisms of *Swietenia mahagoni* seed extract as an antidiabetic agent still remain to be elucidated in experimental studies of diabetic rats, with observation of insulin level, antioxidant status, glycogen content in liver and muscle, as well as the number and condition of beta cells in Langerhans islet of pancreatic tissues, in addition to blood glucose level.

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