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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.

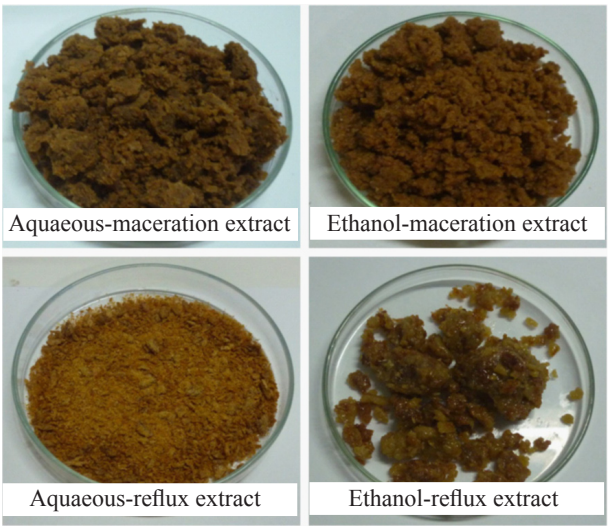

Figure 1. *Swietenia mahagoni* seed extracts.

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*