Ad-Dawaa' Journal of Pharmaceutical Sciences
ISSN: 2654-7392, E-ISSN: 2654-6973
Vol. 4, No. 1, June 2022, 1 – 8
DOI: 10.24252/djps.v4i1.21225

Characterization and Screening Active Phytochemical Compounds of 70% Ethanol Extract of Mahogany Seed (*Swietenia mahagoni* Jacq.)

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ABSTRACT

One of the herbal medicinal plants in Indonesia with antidiabetic activity is mahogany (Swietenia mahagoni Jacq.*). Differences in plant parts used, extraction methods, and solvents during the extraction process will result in different characterization, quantity, and active phytochemical compounds in the extract. This study aimed to determine the content of active phytochemical compounds and extract characteristics of mahogany seed that fit the standards. Extracts were made using the maceration method with 70% ethanol solvent, then extract was characterized, and phytochemicals of active compounds were screened using reagents according to each group of compounds' test method. 50.16 g of 70% ethanol extract of mahogany seeds produced in this study had a solid form, characteristic odor, bitter taste, and brown color. The extract has the characteristics of water content, total ash content, acid insoluble ash content, water-soluble extracts content, and soluble ethanol extracts content according to the standard. Several classes of active compounds were identified qualitatively in the extract, which are flavonoids, phenolics, terpenoids, steroids, glycosides, and saponins. It appears that 70% ethanol extract of mahogany seed has a characterize extract that meets the standards and contains several active phytochemical compounds as potential antidiabetic agents.*

KEYWORDS*: Swietenia mahagoni* Jacq.*, Characterization, phytochemical compounds, 70% ethanol.*

INTRODUCTION

Diabetes Mellitus is a group of diseases caused by carbohydrates, protein, and fat metabolism disorders characterized by hyperglycemia (Wells et al., 2009). Management of diabetes mellitus healing therapy using oral hypoglycemic drugs such as sulfonylurea and biguanides has significant side effects and requires high costs if consumed continuously (Oboh et al., 2017; Sukardiman et al., 2012). These problems have led to an increase in the demand for new antidiabetic natural products with lower side effects and high antidiabetic potential (Ghosh et al., 2011).

Alternative therapy for diabetes mellitus is to consume herbal medicines derived from plants because it has an advantage in terms of safety, and the cost is relatively cheaper (Sukardiman et al., 2012). Herbal medicines are used to treat diabetes, as alternative therapies are reported to have various hypoglycemic effects. Therefore it is necessary to find hypoglycemic drugs that are more safer dan effective in overcoming this problem (Rao & Jamil, 2011).

Mahogany (*Swietenia mahagoni* Jacq.) is one Indonesian medicinal plant that grows

mainly in Java and Sumatra island, famous for its many properties (Ahmad et al., 2019; Hasan, 2017). From the study results, mahogany ethanolic extract can reduce blood glucose levels through the inhibition mechanism of the enzyme alpha-glucosidase, alpha-amylase, phosphodiesterase-5, and arginase (Oboh et al., 2017). Besides, mahogany seeds can increase body weight, the content of the enzyme superoxide dismutase in pancreatic tissue, and reduce the rate of damage from beta-pancreatic cells (Wresdiyati et al., 2015). Tests on humans show that mahogany seeds have a more significant effect on reducing blood glucose levels compared to the antidiabetic drug Glimepiride (Astuti et al., 2017). Research on the search for herbal remedies mostly provides information that the hypoglycemic effect produced by mahogany plants originates from the mechanism of action of active compounds from secondary metabolites such as flavonoid, phenolic, terpenoid, steroid, glycoside, saponin, alkaloid, tannin, or anthraquinone classes contained therein (Ahmad et al., 2019; Moghadamtousi et al., 2013; Oboh et al., 2017).

The making of herbal medicines from several medicinal herbs spread throughout Indonesia generally uses certain plant parts or whole plant parts (Sukardiman et al., 2012). Differences in plant parts, planting conditions, pre or post-harvest processes, and solvents that will be used during the extraction process will result in the quality and quantity of extracts and the content of secondary metabolites contained therein (Jiangseubchatveera et al., 2017; Mostafa et al., 2011; Ratnani et al., 2015). This study aimed to determine the content of active phytochemical compounds as antidiabetic agents and extract characteristics that meet the standards from the seeds of mahogany plants using the maceration method with 70% ethanol solvent as an additional data in the development of herbal medicine products for diabetes mellitus in Indonesia.

MATERIAL AND METHODS

Chemicals and Instrument

Mahogany seeds (*Swietenia mahagoni* Jacq.) obtained and terminated from BPTOOT Tawangmangu (Jln. Raya Lawu No. 11, Tawangmangu), 70% ethanol (PT. Brataco, Indonesia), Toluene (Merck, Germany), Mg powder (Merck, Germany), ether (Merck, Germany), H_2SO_4 (Merck, Germany), $FeCl_3$ (Merck, Germany), ammonia (Merck, Germany), anhydrous acetic acid (Merck, Germany), HCl (Merck, Germany), chloroform (Merck, Germany), benzene (Merck, Germany), NaOH (Merck, Germany), aqua dest, Dragendorff's reagent, Mayer's reagent, analytical scales (Acic AD-2100H, Indonesia), blenders (Miyako, Indonesia), Buchner funnel and vacuum (JOAN Lab, China), rotary evaporator (IKA, Malaysia), oven (Memmert, Germany), and furnaces (Thermoline, Australia).

Preparation of extract

Dried mahogany seeds (*Swietenia mahagoni* Jacq.) are blended using a blender until it forms powder and then dried in an oven at 50˚C for 12 hours. Weight 500 g of powder then macerated in 70% ethanol by ± 5 liters for 24 hours. The filtrate obtained was filtered using a Buchner funnel, and the residue was macerated two times with 70% ethanol, 5 liters each. The maceration filtrate is then dried using a rotary evaporator until a thick extract is obtained.

Extract characterization

Characterization of 70% ethanol extract of mahogany seeds included organoleptic test, water content, total ash content, acid insoluble ash content, water-soluble extract content, and ethanol-soluble extract content. The inspection is carried out following the reference to the determination of standard parameters and test procedures of the Indonesian Herbal Pharmacopeia and Materia Medika Compendium (*Farmakope Herbal Indonesia*, 2008; *Materia Medika Indonesia*, 1980). Each test parameter was replicated to the test three times.

Active phytochemical compounds screening

Flavonoid compound test performed with Shinoda's method, 100 mg dissolved in 10 mL extract, 1 mL solution, and a little Mg powder is added and with 1 mL hydrochloric acid (HCl) (Jiangseubchatveera et al., 2017). Phenolic compound test performed with 100 mg extract and 3 mL ether were put into a test tube, then the ether layer is dried, and the $FeCl_3$ solution is added (*Materia Medika Indonesia*, 1980). Triterpenoid or steroid compound test performed with 100 mg extract was added with three drops of acetic anhydride and one drop of sulfuric acid (H_2SO_4) (Kristanti et al., 2008). Steroid compound test performed with Salkowski reaction method, 100 mg extract is dissolved in 3 mL chloroform ($CHCL_3$) then slowly add a few drops of sulfuric acid (H_2SO_4) (Jiangseubchatveera et al., 2017).

Alkaloid compound test performed with 500 mg extract dissolved 5 mL of 25% ammonia, then added 20 mL of chloroform ($CHCL_3$). The mixture is filtered to obtain a layer of water and an organic solvent layer, and only the water layer is taken. To the water, layers are added two drops of Dragendorff's reagent or Mayer's reagent. With the Dragendorff's reagent, the orange color is formed, and with Mayer's reagent, a white precipitate is formed, indicating the presence of an alkaloid compound (*Materia Medika Indonesia*, 1980). Glycoside compound test performed with 0.1 mL of the extract was evaporated on a water bath and then dissolved in 5 mL of acetic acid anhydrous. Then ten drops of sulfuric acid (H_2SO_4) are added (*Materia Medika Indonesia*, 1980). Saponin compound test performed with 500 mg extract was added with 10 mL of hot aqua dest, then cool and shakes for 10 seconds. The formation of a

stable foam for 10 minutes as high as 1-10 cm indicates the presence of saponin compounds. Add one drop of hydrochloric acid (HCl) 2N, if the foam does not disappear to strengthen the presence of saponin compounds (*Materia Medika Indonesia*, 1980).

Tannin compound test performed with a few drops of $FeCl_3$ were added to 100 mg extract, which has been dissolved in 10 mL of 70% ethanol (Jiangseubchatveera et al., 2017). Anthraquinone compound test performed with 200 mg extract was added with 2N sulfuric acid (H_2SO_4), heated briefly after a cold 10 mL of benzene was added. Take the benzene layer then mix with 0.2 mL of 2N NaOH. The red aqua dest layer and the colorless benzene layer show anthraquinone (*Materia Medika Indonesia*, 1980).

RESULTS AND DISSCUSSION

The extracts in this study were carried out by the maceration method by soaking mahogany seed powder (*Swietenia mahagoni* Jacq.) with 70% ethanol. With this simple method, the resulting extract is as much as 50.16 g or 10.03% w/w extract yield. The use of 70% ethanol solvent affects the thick extract produced, the addition of water in the solvent will increase the polarity of the solvent and the speed of the release of bonds between compounds and plant simplicia that more compounds dissolve while increasing the amount of thick extract produced (Kumoro et al., 2009).

The result from extract characterization in Table 1. shows that ethanol extract of 70% mahogany seeds has a characteristic organoleptic form that is thick, characteristic odor, bitter taste, and brown. The consistency of the extract form will be influenced by the value of the percentage of water content. In this study, the value of the extract's water content is 7.24±0.09% v/w. The water content in traditional medicine preparations, including extracts, should not exceed the 10% limit because it can cause the extract to be quickly overgrown with fungi (*Materia Medika Indonesia*, 1980).

Testing the total ash content of the extract was carried out by incandescent the extract at a temperature of 600ºC in the furnace to a constant weight (*Farmakope Herbal Indonesia*, 2008). In this process, organic compounds and their derivatives will be decoded so that only the mineral content remains. The purpose of determining the total ash content is to know the description of the total amount of material remaining after incubation such as inorganic compounds, metals, residues of compounds outside plants (sand or soil attached). In contrast, the acid insoluble ash content test which is a continuation of the determination of total ash content has the aim to determine the high content of metals which are insoluble in acids and silicates derived from sand or soil from extracts (*Farmakope Herbal Indonesia*, 2008; Ratnani et al., 2015). Obtained average test results for total ash content and acid insoluble

Table 1. Characterization of the extract results

Testing Parameters	Results
Organoleptic	Solid form, characteristic odor, bitter taste, and brown color
% yield extract	10.03
Water content	7.24 ± 0.09 % v/w
Total ash content	9.14 ± 0.04 % v/w
Acid insoluble ash content	0.26 ± 0.08 % v/w
Water-soluble extract content	31.15 ± 0.10 % v/w
Ethanol-soluble extract content	34.43 ± 0.15 % v/w

Note: (% v/w) percentage of volume per weight ; (% w/w) percentage of weight per weight

ash content of mahogany seed extract that is equal to 9.14±0.04% w/w and 0.26±0.08% w/w. The value of total ash content and acid insoluble ash content is following the value of determining the required standard value of less than 10.20% w/w and 1.71% w/w so that it still meets the standards of the Indonesian Herbal Pharmacopeia (*Farmakope Herbal Indonesia*, 2008; Handayani et al., 2019).

The test value of water-soluble and ethanol extracts is an indicator of the level of active compounds, namely secondary plant metabolites, which can be found or dissolved in extracts, either using water or ethanol solvents during the extraction process. The level of active compound in an extract is influenced by plant age, harvest time and climate, and place of growth (*Farmakope Herbal Indonesia*, 2008; Ratnani et al., 2015). The test results showed that the average value of extract of water-soluble mahogany seeds was 31.15±0.10% w/w, and ethanol soluble was 34.43±0.15% w/w. These results are sufficient to indicate that secondary metabolite compounds in 70% ethanol extract of mahogany seeds are easily found or dissolved in water and ethanol (Ratnani et al., 2015).

Phytochemical screening of active compounds from secondary metabolites that have been carried out has resulted in several classes of compounds in the extract shows in Table 2. Samples of thick mahogany seed extract showed that there were flavonoids in it that were proven by the appearance of a brownish-red colour after testing the content of flavonoid compounds (Jiangseubchatveera et al., 2017). Flavonoids are included in essential compounds of several other secondary metabolites which have antioxidant and antidiabetic activity in vitro (Sarian et al., 2017). Bluish purple indicates the presence of phenolic compounds in the sample. Flavonoids are included in the class of polyphenolic compounds in chemical structure, so the presence of flavonoid compounds in the extract will also trigger positive results indirectly (Sarian et al., 2017). In addition to the two classes of compounds, saponin is also contained in a concentrated extract seen from foam formation as high as 1-10 cm, which is stable for 10 minutes and

Table 2. Screening active phytochemical compounds of the extract results

Active compounds	Results
Flavonoid	+
Phenolic	+
Terpenoid	+
Steroid	+
Alkaloid	-
Glycoside	+
Saponin	+
Tannin	-
Antraquinon	-

Note: (+) identified; (-) not identified

does not disappear after adding 2N HCl (Kristanti et al., 2008; *Materia Medika Indonesia*, 1980).

Triterpenoid class terpenoids were detected in samples seen from the appearance of a rather faint blue (Kristanti et al., 2008). The red color's appearance in the Salkowski's reaction test indicates the presence of steroid compounds in the sample (Jiangseubchatveera et al., 2017). Stigmasterol and sitosterol-3-O-β-D-glucopyranose are examples of steroid compounds. These compounds can reduce random blood sugar (GDA) of rats with diabetes by increasing the amount of serum insulin, especially at a dose of 0.50 mg/kg BW. This effect of decreasing random blood sugar (GDA) is likely to occur due to the regeneration of beta-pancreatic cells that have been damaged by streptozotocin (Ahmad et al., 2019; Nualkaew et al., 2015). Besides the hypoglycemic effect of this compound, it also increases the value of biochemical parameters, hematology, cholesterol levels, HDL-C, ALT, AST, and bilirubin levels to prevent complications diabetes and improve the function of the kidneys and liver (Nualkaew et al., 2015; Puttaswamy & Urooj, 2016). Nontoxic fractions of mahogany seeds are also reported to have hypoglycemic activity with the insulonomimetic agents' mechanism and enhancers of insulin synthesis (Naima et al., 2017).

The last compound detected in the sample was glycoside. These results were obtained after the appearance of a blackish-blue color in glycoside compounds' test content (*Materia Medika Indonesia*, 1980). Limonoids and their derivatives are a group of glycoside compounds being the main constituents of mahogany (Moghadamtousi et al., 2013). Methanol extract from mahogany seeds identified by spectroscopy produced two limonoids, namely swietenolide and 2-hydroxy-3-O-tigloylswietenolide (Rahman et al., 2009). Swietenin on the seeds of mahogany has a hypoglycemic activity (Maiti et al., 2009). Alkaloid, tannin, and anthraquinone compounds do not produce positive results in the sample. The absence of these three compounds may be due to the use of different parts of plants and solvents during extraction, and the extraction method has the type or amount of content of secondary metabolites contained therein (Jiangseubchatveera et al., 2017; Wresdiyati et al., 2015). This statement is supported by previous research that mahogany seed water extracts contain saponin and triterpenoid compounds, but do not contain alkaloid,

steroid, tannin, and anthraquinone compounds (Wresdiyati et al., 2015).

CONCLUSION

70% ethanol extract of mahogany seed has a characterize extract that meets the standards and contains several class of active phytochemical compounds.

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