



## Standardization and bacteria inhibitory test of purified extract of mahogany (*swietenia mahagoni* (L.) Jacq) seeds and leaves

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### ABSTRACT

Mahogany (*Swietenia mahagoni* (L.) Jacq) is one of the plants that is often used by the community as traditional medicine. One of them is antifungal, antibacterial, antidiabetic, and eczema. Standardization of purified extract of mahogany has been carried out according to the monographs of extract standardization guidelines, which include testing of specific and non-specific parameters. The results of the specific parameter testing showed that the purified extract of mahogany seeds is a thick extract, brown to reddish, smells distinctive and has a bitter taste. While the purified extract of mahogany leaves is a thick extract, greenish-brown in color, distinctive smell and has a bitter taste. The chemical content of purified extract of mahogany seeds and leaves showed the presence of flavonoids, alkaloids, terpenoids and saponins. Water-soluble essence levels in mahogany seeds and leaves was 14.84% and 10.28%. While the ethanol-soluble essence levels in mahogany seeds and leaves were 15.38% and 12.43%. Testing of non-specific parameters on mahogany seeds and leaves showed the results of drying shrinkage levels of 0.22% and 8.84%, moisture content of 2.60% and 4.04%, total ash content of 1.71% and 1.93%, levels acidic insoluble ash 0.38% and 0.32%, Total Plate Number (ALT) of mahogany seed bacteria  $1 \times 10^2$  colonies/g, Number of mahogany mold seeds  $4 \times 10$  colonies/g, heavy metal lead contamination and cadmium in mahogany seeds  $0.0607 \mu\text{g/g}$  and  $<0.003 \mu\text{g/g}$ . The inhibitory diameter of each concentration of leaves against *Escherichia coli*, 3%, 5%, 7%, and 9%, is 10,27; 10,90; 13,46; and 15,68 mm, respectively.

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### INTRODUCTION

In Indonesia, there are more than 30,000 species of plants and more than 1000 kinds of herbs that have been used in the traditional medicine industry (Day, 2004). Medicinal plants in Indonesia increasingly utilized both as an Indonesian traditional medicine (herbal medicine), or any standardized herbal medicine as phytopharmaca. Various research and development that take advantage of technological advances, as well as part of efforts to improve the quality and safety of products, are

expected to further increase confidence in the benefits of natural medicine (Anam *et al.*, 2013).

Traditional medicine is made in the form of the extract to give the maximum effect compared to use in the form of botanicals. The extract can be in the form of dry extract, extract viscous and liquid extract.

Mahogany (*Swietenia mahagoni* (L.) Jacq) is a plant that grows in the tropics and is widely used as a protective plant also have medicinal properties. Mahogany has empirically been used by the community as a diabetes drug (Rasyad *et al.*, 2012).

Seeing the tremendous potential of mahogany (*Swietenia mahagoni* (L.) Jacq) seeds and leaves as a medicinal plant, it is necessary to standardize and bacteria inhibitory test from purified mahogany seeds and leaves extract so that it can set the quality, safety and quality of raw materials extracts. Standardization is also done so that the user can receive and develop as one of the Indonesian native plants that can be used as ingredients for traditional medicines.

## MATERIALS AND METHODS

### Sampling and processing

Sample of mahogany (*Swietenia mahagoni* (L.) Jacq) seeds that has been obtained is cleaned and dried in the drying cabinet and then pulverized and prepared for extraction.

### Extraction and sample purification

Dry simplicia that has been mashed and then weigh the seeds and leaves each as much as 3.750 g and 1,500 g then put into containers to be macerated separately. Added 6 liters of ethanol 96% respectively into the receptacle containing the powder grains and leaf. Then macerated for 3 days while occasionally stirred, and then macerated again until the solvent is turned into translucent. Furthermore, filtered into new containers in order to obtain the liquid extract. Results of the extract were evaporated by using a rotary vacuum evaporator to obtain a viscous extract.

Viscous 96% ethanol extract from seed and leaf of mahogany (*Swietenia mahagoni* (L.) Jacq) were weighed every 30 grams then inserted into the separation funnel and add n-hexane  $\pm 100$  mL. Shake occasionally for 3-5 minutes with the lid open. Then allowed to stand to form two layers, the top layer is n-hexane soluble phase, and the bottom layer is insoluble n-hexane phase. Insoluble n-hexane phase inserted again into the funnel. Done repeatedly until the late phase of n-hexane looks colorless. Insoluble

n-hexane phase-separated and evaporated again.

### Specific Parameter Determination

#### Parameter extract identity

Parameter extract identity is done by providing the objective identity of the plant names that include a description of nomenclature extract, names of plants, plant parts used (D K R Indonesia, 2000).

#### Organoleptic

The organoleptic test is a simple initial introduction to the sample. Organoleptic tests carried out by observation of the shape, color, smell and taste (D K R Indonesia, 2000).

#### Water-Dissolving Test

Each of the purified extract of the mahogany (*Swietenia mahagoni* (L.) Jacq) leaf and seeds weighed as much as  $\pm 5.0$  g, macerated for 24 hours with 100 ml of water-chloroform using closed- flask while repeatedly shaken during the first 6 hours and then left to stand for 18 hours, then filtered. The filtrate obtained was evaporated up to 20 mL into the cup which has settled. The residue is heated at a temperature of 105<sup>o</sup> C until the weight is constant. Count the levels of percentage of the water-soluble compound to the weight of the initial extract (D K R Indonesia, 2000).

#### Ethanol-Dissolved Test

Each of the purified extract from the seeds and leaf of Mahogany (*Swietenia mahagoni* (L.) Jacq) weighed as much as  $\pm 5.0$  g and macerated for 24 hours with 100 mL of ethanol (95%) use closed-flask while repeatedly shaken for 6 hours first and then left to stand for 18 hours. Filtered quickly to avoid evaporation of ethanol, 20 mL of the filtrate is then evaporated to dry then put into a cup which has settled, the residue heated at a temperature of 105<sup>o</sup>C until the weight is constant. Count the levels of percentage compound soluble in ethanol to the weight of the initial extract (D K R Indonesia, 2000).

#### Test chemical constituents extract

##### Alkaloid Test

The mobile phase was made by using n-butanol: acetic acid: water (8:1:1) for purified extract mahogany seeds and n-hexane: ethyl acetate (6:4) for the purified extract mahogany leaves. Then put in a separate chamber and allowed to saturate. Spotted on a TLC plate respectively and the plate was added to the chamber, eluted until the limit mark, taken and allowed to dry. Then observed spots under UV light and sprayed with Dragendorff reagent. It positive would contain alkaloids if the colour brown or orange-brown appeared after

sprayed with Dragendorff reagent.

### Flavonoid Test

The extract contains flavonoids if viewed under UV light 366 nm. The spots appeared with green/blue-colored fluorescence or yellow if using Sitroborat reagent and positive contain flavonoid if red-orange or pale yellow appeared using AlCl<sub>3</sub> reagent.

### Terpenoid Test

Then observe the spots under UV light and sprayed with a reagent Vanillin-sulfuric acid, and then heated at a temperature of 120°C for 5 minutes. The extract contains terpenes if the dots appear brown (Suliman, 2018).

### Saponin Test

Each purified extract was weighed as much as 1 gram using an analytical scale and then put into a test tube. After that, put 10 mL of hot water into the tube then shake vigorously. The extracts positively contain saponin if the sample formed foam, and after spilled with HCl 2N, the foam is not disappeared (Ahmad et al., 2014).

### Specific Parameter Determination

#### Determination of drying shrinkage

Each purified extract weighed as much as 1-2 grams and inserted into the cup previously been heated at a temperature of 105°C and settled. Before weighed, flattened extract in a bowl to form a thick layer approximately 5 mm to 10 mm, and then put into an oven at 105°C for 30 minutes until the weight remains. Then, let the cup in exicator until cool to room temperature. Then weighed and counted percent of drying shrinkage (D K R Indonesia, 2000).

#### Water content

##### Gravimetric method

Each purified extract weighed as much as ±10 g in a porcelain dish that has settled. Then dried at 105°C for 5 hours in the furnace, then enter into exicator until it reaches room temperature and weighed. Then calculate the percentage of water content in the sample (D K R Indonesia, 2000).

#### Ash Total

Purified extract each weighed as much as ±2-3 grams and then put into a porcelain dish that has been heated and settled, then inserted into the furnace at a temperature of 500-550°C until the charcoal run out. Then remove and inserted into exicator to cooled until room temperature. Weighed and count the percent of the total ash content in the sample (D K R Indonesia, 2000).

#### Acid-Insoluble ash levels

Ashes obtained from each seed and leaf mahogany (*Swietenia mahagoni* (L.) Jacq) ash assay were boiled with 25 ml of aqueous sulfuric acid for 5 minutes, acid-insoluble part was collected and filtered using ash-free filter paper, after that, wash with hot water and then heated to a fixed weight and weighed. Then, count the ash content that is not soluble in acid (D K R Indonesia, 2000).

#### Microbial contamination

Purified extract from seeds of mahogany (*Swietenia mahagoni* (L.) Jacq) weighed as much as 1 gram dissolved in 10 mL of solvent, then shaken until homogeneous to obtain a dilution of 10<sup>-1</sup>. Prepared 4 test tube, and included 9 mL of diluent to each tube. Pipette in 1 mL of diluent 10<sup>-1</sup> into the first test tube, shaken until homogeneous to obtain a dilution of 10<sup>-2</sup>, then proceed with the dilution of 10<sup>-3</sup>, 10<sup>-4</sup> and 10<sup>-5</sup> (Handayani et al., 2019).

#### Total plate count (TPC) bacteria

From each dilution, 1 mL was pipetted into petri dishes using a different and sterile pipette. Then, into each Petri dish poured 15 ml of medium Nutrient Agar that has been melted and the petri dish was shaken so that the suspension is well blended. After that, left to solidify the mixture in a petri dish. Petri dishes were placed upside-down position and then inserted into the cabinet incubator at 37°C for 24 hours. Then, observed and counted the number of colonies that grow.

#### Mold and Yeasts Rate

From each dilution, 1 mL was pipetted into petri dishes using a different and sterile pipette. Then, into each Petri dish poured 15 ml of Potato Dextrose Agar (PDA) medium. Then, the PDA medium, which is still liquid suspension, was shake until the suspension mixed well. After that, incubated at a temperature of 25°C for 3 days. Then observed and counted the number of colonies that grow.

#### Heavy metal contamination

Atomic Absorption Spectrophotometer (AAS) (Lead (Pb) and Cadmium (Cd))

Prepared samples of destruction then add with aqua dest 50 mL, then measured the atomic absorption spectrophotometry with a wavelength of 217 nm to 228.8 nm for lead and cadmium (Makanan, 2014).

#### The Antibacterial Activity Test

The antibacterial activity test of Mahogany (*Swietenia mahagoni* (L.) Jacq) seed and leaves against *Escherichia coli* was determined by using the diffusion method. The liquid medium of Nutrient Agar was poured into sterile Petri for 20 ml and waited until become solid. Once the Agar become solid, 100

$\mu\text{l}$  bacterial suspension was spread on the Agar. The solute test in each 10  $\mu\text{l}$  was dropped into the sterile disk, and then, it put on the Agar. Subsequently, the sterile Petri cup was incubated in the reverse position at 37°C for 48 hours (Handayani and Wisdawati, 2015).

## RESULTS AND DISCUSSION

Standardization in the pharmacy is none other than a series of parameters, procedures and measurements in related elements of pharmaceutical paradigm, in terms of qualified quality standards (chemical, biological, and pharmaceutical), including the guarantee limits of stability as pharmaceutical products generally. In other words, the notion of standardization also means the process ensures that the final drug product (drug, extract, or the extracted product) has a constant value of certain parameters and predetermined (D K R Indonesia, 2000).

This research aims to establish the parameters of standardization of purified extract from mahogany (*Swietenia mahagoni*(L.) Jacq) seeds so that the future can provide scientific information to ensure the final product and its use can be accepted and can be developed as one of the Indonesian native plants that can be used as ingredients for traditional medicines (Handayani et al., 2019).

Mahogany seeds were obtained from the city of Bogor, West Java. While mahogany leaf was obtained from the city of Makassar, South Sulawesi. After the sample pulverized and then extracted using cold methods called maceration. Maceration method was chosen as the method of extracting, hoping that it won't damage the chemical components contained in the sample due to the lack of heating in the extraction process. In addition, the maceration process can be done in a simple way and using easy tools. In this study, the solvent used is ethanol because it has properties that can dissolve almost all substances, both polar and non-polar and its absorption is good and relatively low toxicity levels.

Results obtained from the seeds and leaves of mahogany after extraction then collected and concentrated by using a rotary vacuum evaporator until a viscous ethanol extract. Then the ethanol extract of the seeds and leaf purified using n-hexane solvent. The advantage of purifying itself can eliminate chemical component or unwanted impurities that can interfere instability or extract so as to increase the concentration of the active compound and reduce the mass or volume of extract. Because of the presence of such substances do more harm

instability and reduce the levels of the active compounds in the extract, so we need purification. Solvent n-hexane is used as a solvent because it has low polarity so that it will attract the chemical components that are less polar such as lipids, wax, etc. (Anam et al., 2013). Then the resulting extract is evaporated to remove the remains of n-hexane, contained in the extract until thick. So, in this study showed yield value of two types, namely ethanol extract and purified ethanol extract, which can be seen in Table 1.

The data above shows that the yield of ethanol extract of seeds of mahogany that is 5.83% and the yield of purified extract mahogany seeds as much as 33.79% (Handayani et al., 2019). While the value of the yield of ethanol extract of leaves of mahogany that is 10.67% and the yield of purified extract of leaves of mahogany as much as 69.61%. As for purposes of calculating the percent yield is to determine the number of compounds that are pulled in a particular solvent but cannot be the type of compounds that are carried away.

Extract identity checks aim to provide as objective as possible the identity of the plant used (D K R Indonesia, 2000). As seen in Table 2 that the results of identification show that the sample is completely plant mahogany species *Swietenia mahagoni* (L.) Jacq. With parts used are the seeds and leaves. This is evidenced by the determination of plant mahogany done beforehand to determine typical characteristics.

The organoleptic evaluation determined using the five senses and aim for the early introduction, simply and subjectively (Arifin et al., 2006). On examination of the organoleptic testing directly to shape, color, smell and taste of a purified extract of the seeds and leaves of mahogany. From observations showed that the consistency of purified extract mahogany seeds has a thick, brown to reddish, with a specific odor and bitter taste while the results of a purified extract of leaf mahogany has thick, greenish-brown consistency, with a specific odor and bitter taste.

Testing of compounds that are soluble in certain solvents, which are water and ethanol with the aim of estimating the content of compounds which are soluble in water and ethanol. From the results of research, seeds and leaves of mahogany obtained water-soluble compounds as much as 14.84% and 10.28%. While ethanol-soluble compounds in seeds and leaves of mahogany are 15.3818% and 12.43%. The results of identification of chemical content showed that purified extracts of mahogany seeds and leaves contain alkaloids, flavonoids, terpenoids



**Table 1: Yield Value of Seeds and Leaf from Mahogany (Swieteniamahagoni(L.)Jacq)**

No	Samples	Type Extract	Sample (g)	Extract (g)	Yield Value (%)
1	Seed	Ethanol	3.750	218,60	5,83
		Purified	180,04	60,83	33,79
2	Leaf	Ethanol	1.500	160,03	10,67
		Purified	120,17	83,65	69,61

**Table 2: The result of the standardization of specific parameters**

No.	Examination	Result	
		Seed	Leaf
1	Identity extract	<i>Swietenia mahagoni (L.)</i>	<i>Swietenia mahagoni (L.)</i>
	Latin name	Jacq Seed	Jacq Leaf
2	Plant parts	Thick	Thick
	Appearance test	Brown to reddish	greenish brown
	Form	Typical	Typical
	Color	Bitter	Bitter
	Smell flavor		
3	Levels of water soluble extract	14.84%	10.28%
4	Levels of soluble extract ethanol	15.38%	12.43%
5	Identification of chemical constituents	alkaloids, flavonoids, terpenoids, and saponins	alkaloids, flavonoids, terpenoids, and saponins

and saponin.

The results of non-specific standardized testing can be seen in Table 3. Where the Drying shrinkage testing aimed to provide maximum limits (range) of the amount of the compound is lost in the drying process. Parameter drying shrinkage is basically a measurement of residual substances after drying at 105°C for 30minutes. At 105oC temperature of the water will evaporate, and the compounds that have a lower boiling point than water will also evaporate well (D K R Indonesia, 2000). The results of the determination of drying shrinkage on purified extract the seeds and leaf mahogany (*Swietenia mahagoni (L.) Jacq*) obtained 0.22% and 8.84%.

Measurement of water content in an extract aims to provide a minimum limit or range of the amount of water content in the materials (extracts). According to the literature, the water content in the extract should not be more than 10%. The high-water content can result in the growth of bacteria and fungi that are not good for health (D K R Indonesia, 2000). The results of water content obtained in purified extracts of seeds and leaves of mahogany 2,60% and 4,04%, which means it meets the requirements set out in the literature.

Determination of ash content aims to provide an

overview of internal and external mineral content originating from the beginning to the process of formation of the extract. At this stage in the heated to extract organic compounds and derivatives destructed and evaporated to stay and inorganic mineral elements only. Total ash from the purified extract of the seeds and leaf mahogany gained by 1.71% and 1.93%. While the levels of acid-insoluble ash of 0.38% and 0.32%. The amount of total ash content in the purified extract seeds mahogany (*Swietenia mahagoni (L.) Jacq*) showed that the samples contained minerals. As for the assay of acid-insoluble ash was intended to evaluate the contamination of extract-containing materials such as soil and sand.

Testing Total Plate Count (TPC) bacteria and Yeast-Mold Numbers in order to provide assurance that the extracts may contain microbes exceeds the set limit because it can affect the stability of the extract and dangerous (toxic) for health. Microbial contamination testing that has been done shows that the purified extract seeds mahogany (*Swietenia mahagoni (L.) Jacq*) has a value of ALT bacteria  $1 \times 10^2$  colonies / g and the numerical value of yeast fungi of  $4 \times 10$  colonies/g, which means it meets the requirements set out in the literature. That the ALT value  $\leq 10^4$  bacterial colonies/g and the number of yeastfungi  $\leq 10^3$  colonies/g (Handayani et al.,

**Table 3: The result of the standardization of non-specific parameters**

No.	Examination	Result	
		Seed	Leaf
1	Drying shrinkage	0.22%	8.84%
2	Water content	2.60%	4.04%
3	Ash Total	1.71%	1.93%
4	Ash Insoluble-Acid Levels	0.38%	0.32%
5	Microbial contamination	1 x 10 <sup>2</sup> colonies / g	-
	Total plate count bacteria	4 x 10 colonies / g	-
	Figures mold yeasts		
6	Heavy metal contamination	0.0607 pg / g	-
	Lead (Pb)	<0.003 ug / g	-
	Cadmium (Cd)		

2019).

Examination of heavy metal contamination in the form of metallic lead (Pb) and Cadmium (Cd). The level of heavy metals lead (Pb), and cadmium (Cd) on extracts used to ensure that the extract does not contain lead exceeding the established limits for toxic to the body. According to SK Badan POM Nomor12 about maximum limit metal contamination in food, states that the maximum limit metal contamination of lead (Pb) and cadmium (Cd) on at  $\leq 10$  mg / g to metallic lead (Pb), while the metal cadmium by  $\leq 0,3$  ug / g (BPOM RI, 2014). From the metal, content measurement showed that the purified extract mahogany seeds had higher levels of 0.0607 ug / g to metallic lead (Pb) and <0,003 ug / g for metal cadmium (Cd) (Handayani *et al.*, 2019).

The inhibitory diameter of each concentration of seeds against *Escherichia coli*, 3%, 5%, 7%, and 9%, is 12,76; 13,67; 17,67; and 19,67 mm, respectively. The inhibitory diameter of each concentration of leaves against *Escherichia coli*, 3%, 5%, 7%, and 9%, is 10,27; 10,90; 13,46; and 15,68 mm.

## CONCLUSION

Based on research that has been done then the data showed that the purified extract the seeds and leaf mahogany (*Swietenia mahagoni* (L.) Jacq) is a thick, brown to reddish and greenish-brown extract, has a specific odor and have a bitter taste. Test chemical constituents purified extract the seeds and leaf mahogany indicate flavonoids, alkaloids, terpenoids, and saponins. With water-soluble compounds 14.84% and 10.28%, a compound soluble in ethanol 15.38% and 12.43%, drying shrinkage of 0.22% and 8.84%, 2.60% and a water content 4.04%, total ash content of 1.71% and 1.93%, acid insoluble ash content of 0.38% and 0.32%, microbial contamination in grain mahogany covering total

Plate Count (TPC) of bacteria 1 x 10<sup>2</sup> colonies / g, Yeast Molds figure of 4 x 10 colonies / g, heavy metal contamination of lead (Pb) in mahogany seeds of 0.0607 mg / g and cadmium (Cd) <0.003 ug / g. The extract of Mahogany (*Swietenia mahagoni*(L.)Jacq)seed and leaves has antibacterial activity against *Escherichia coli*.

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