COMPARISON OF TOTAL FLAVONOID CONTENT BASED ON DIFFERENCES IN ETHANOL SOLUTION CONCENTRATION FROM MAHOGANY FRUIT SEEDS (*Swietenia mahagoni*)

Katharine Nathasa[1*], Ali Rakhman Hakim[1] and Nurul Hidayah[2]
[1]*Departement of Pharmacy, Health Faculty, Sari Mulia University, Indonesia*
[2]*Departement of Health Promotion, Health Faculty, Sari Mulia University, Indonesia*
**knathasa@gmail.com*

Abstract
Mahogany is one of various kinds of plants that empirically can be used by the community for various treatments such as diabetes and hypertension, especially people in Indonesia. extraction test was carried out using ethanol solvents with different concentrations of 70% ethanol and 96% ethanol to determine the most suitable solvent concentration to obtain total flavonoid. The purpose of this study was to determine the total flavonoid content of mahogany (Switenia mahagoni) seeds based on differences in ethanol solvent concentrations. This research used observational research methods. Observation is a method of collecting data by conducting systematic observations and recordings either directly or indirectly at the place being observed. Total flavonoid content of each mahogany seed sample was obtained, namely in 70% ethanol 2.138% while in ethanol 96% 0.109% where the total flavonoid content in 70% ethanol was higher. Based on the results of research that has been carried out, it can be concluded that 70% and 96% ethanol extracts contain flavonoid secondary metabolites and from the measurement results of total flavonoid levels, 70% solvent is more effective in extracting flavonoid compounds.

Keywords: Flavonoids, Mahogany seeds, Total Flavonoid content.

Introduction

Indonesia is a country that has a variety of natural resources with various types of plants that are efficacious for traditional medicine. Traditional medicine is in demand by many people because the vegetable ingredients are easy to find, easy to process, and the price is affordable, so the quality and quality of the natural ingredients used must be improved to suit the community's needs. Medicinal plants have been used since the time of our ancestors (Nusantara) which are used to maintain health and treat various diseases. The knowledge from these ancestors is passed down from generation to generation but has not been tested scientifically to determine the chemical content contained in the plant (Ulfah Diana et al, 2016). One of the medicinal plants that are interesting to study is the seeds of mahogany

fruit from the Meliaceae family. Mahogany (*Swietenia mahagoni*) is a plant known to have various properties as traditional medicine; mahogany plants can also grow well in AC-type climates; mahogany also has relatively fast growth, only in the range of 7 to 15 years, mahogany plants can be harvested (Haikal, 2010). Extracts from mahogany seeds in previous studies stated that they contained various secondary metabolites, one of which was flavonoids which were proven to have antioxidant activity (Roni and Hanny 2016). Flavonoids are one of the compounds with the most influential natural phenol group found in all green plants so that they can be found in every extract from plants. Flavonoids are a class of compounds that are widely presented in nature. Flavonoids are also included in the water-soluble polyphenol family. The polyphenol group is a group that is known to have properties as free radical scavengers or as antioxidants and can inhibit hydrolysis, oxidative enzymes, and if further processed can work as an anti-inflammatory (Hui Cao et al, 2015). To obtain flavonoid compounds, it is necessary first to extract the seeds of mahogany fruit (*Swietenia mahagoni*). Extraction methods that can be done include maceration, percolation and soxhletation. The extraction method in this study uses the maceration method. The suitable solvent for extracting flavonoid compounds from mahogany (*Swietenia mahagoni*) seeds is ethanol. Ethanol is the solvent most often used for the extraction process because ethanol does not have a toxic effect compared to acetone and methanol solvents, the cost is relatively cheap, easy to obtain, efficient, safe for the environment, and the extraction rate is relatively high (Hakim & Saputri, 2020). Several factors that affect the extraction rate are the type of extraction, temperature and type of solvent. Ethanol is the solvent most often used for the extraction process of flavonoid compounds because of its polar nature (Arifin et al, 2006; Utami, 2009; Hanani, 2016; Widarta and Arnata, 2017). In addition to the type of solvent used, the difference in solvent concentration also affects the extraction results. Therefore, a mahogany (*Swietenia mahagoni*) seed extraction test was carried out using ethanol solvents with different concentrations of 70% ethanol and 96% ethanol to determine the most suitable solvent concentration to obtain total flavonoid compounds from mahogany (*Swietenia mahagoni*) seeds and to see The results of the total flavonoid compounds

contained in the seeds of mahogany fruit were used by the UV-Vis spectrophotometer method.

Material and Methods

This research is a research using experimental method. Sampling of mahogany seeds was carried out in the Tamiang Layang area, Central Kalimantan. The fruit taken is fruit that has ripened and falls by itself from the tree. Analysis of flavonoid content was carried out to see whether this mahogany seed extract contained flavonoids. Several reagents were used to see the content of flavonoids such as FeCl3 and concentrated HCL reagents. In this study, the extract was tested using these two reagents. To ensure that the color contained in the test tube does indeed indicate the content of flavonoids, another TLC test or thin layer chromatography was carried out by spraying the reagent on the TLC plate.

a. Sampling

This research is research using an experimental method. A sampling of mahogany seeds was carried out in the Tamiang Layang area, Central Kalimantan. The fruit taken is the fruit that has ripened and falls by itself from the tree. Analysis of flavonoid content was carried out to see whether this mahogany seed extract contained flavonoids. Several reagents were used to see the content of flavonoids such as FeCl3 and concentrated HCL reagents. In this study, the extract was tested using these two reagents. To ensure that the colour in the test tube does show the flavonoid content, another TLC or thin layer chromatography test is carried out by spraying the reagent on the TLC plate.

b. Extraction

The samples of mahogany (*Swietenia mahagoni*) seeds that have been collect are being sorted by wet sorting. After that, wash with running water and dry in the sun. Puree the mahogany seeds using a blender. Dry sorting, Then put it in a jar and store it at room temperature. Weighteach 200 g of simplicia seeds of mahogany (*Swietenia mahagoni*), put them in vessels 1 and 2. Put 70% ethanolsolvent into maceration vessel 1 and enter 96% Ethanol solvent into maceration vessel 2 until this filter solution soaks 1cm an oven the powder-face% Cover the vessel tightly with aluminum foil. Store the maceration vessel for 24 hours in a place away from sunlight and still the extract occasionally. Within 3 x 24 hours, filter the simplicia filtrate and enter the filtrate into the container. Then put the same solvent back into each maceration vessel and repeat until a dry extract is obtained. Evaporate each liquid extract in a water bath at 60°C until a thick extract is obtained.

c. Analysis of Flavonoid Content

Analysis using FeCl3 reagent was carried out by weighing 1 mg each of the thick ethanolic extract of mahogany seeds *(Swietenia mahagoni)* from vessels 1 and 2. Then put it into the t-test tube. Add 2 drops of FeCl3 into each test tube. If the color changes to blue, it indicates the presence of flavonoids in the extract.

Analysis using magnesium powder was carried out by weighing 1 mg each of the thick ethanol extract of mahogany seeds *(Swietenia mahagoni)* from vessels 1 and 2. Then put it into a test tube. Add 0.05

mg of magnesium powder to each test tube. Add 1 ml of concentrated HCL into each test tube. If there is a change in color to yellow, it indicates the presence of flavonoids in the extract.
Analysis using TLC was carried out by making a mobile phase consisting of n-hexane: ethyl acetate (3:7). Put it in the chamber and leave it until it is saturated. Prepare silica gel 20cm x 20cm, then mark 1cm at the bottom and 1cm at the top of the silica gel. Sprinkle each extract that has been diluted with ethanol at the bottom of the silica gel, then put into the chamber. Elute to the upper limit mark. Take it and dry it. Then viewed under UV light 254 nm and UV 366 nm. Detection with the appearance of the FeCl3 spot will show a blue-black color, Sitroborate will show a greenish yellow color and Ammonia will show a yellow-brown color (Harbone, 1996)

d. Determination of Total Flavonoid Content

To determine the maximum wavelength of quercetin, it must be done by running the quercetin solution first in the wavelength range of 400 - 450nm. This maximum wavelength was used to measure the absorption of the ethanol extract sample of mahogany (*Swietenia mahagoni*) seeds.
Preparation of the Quercetin Standard Curve was carried out by weighing 25 mg of the quercetin standard for solutions 1 and 2. Dissolve it into 25 mL of 70% ethanol and 96% ethanol, respectively. Each stock solution was pipetted as much as 1 mL ad 10 mL with ethanol to obtain a concentration of 100 ppm.
Determination of Operating Time is done by steps of a standard standard solution of 100 ppm quercetin taken as much as 1 mL. Add 1 mL of 2% AlCl3 and 1 mL of 120 mM potassium acetate. The absorbance of the solution was measured at the maximum wavelength in the span of 5 minutes, 10 minutes, 15 minutes, 20 minutes, 30 minutes and then averaged. Observing the relationship curve between absorbance, time, and determine the operating time (Ipandi, Triyasmono, & Prayitno, 2016).
Determination of the quercetin standard curve, namely the quercetin parent standard solution of 100 ppm quercetin made in several concentrations of 6 ppm, 8 ppm, 10 ppm, 12 ppm and 14 ppm. A total of 1 mL of the concentration series solution was reacted with 1 mL of 2% AlCl3 and 1 mL of 120 Mm potassium acetate. Leave it for operating time. The absorbance was measured at the maximum wavelength obtained (Ipandi et al., 2016).
Determination of Total Flavonoid Content Using UV-Vis Spectrophotometry was carried out by weighing 15 mg of simplicia extract each. The first extract was dissolved in 10 mL of 70% ethanol and the second extract was dissolved in 10 mL of 96% ethanol, so that each concentration was 1500 ppm. Pipette 1 mL each, put into a test tube. Add 1 mL of 2% AlCl3 solution and 1 mL of 120 mM potassium acetate into each test tube. Incubated during operating time and at room temperature. The absorbance was determined using UV-Vis spectrophotometry method with a maximum wavelength of 435 nm. Each sample was made in three replications. for each analysis and obtain the average value of absorbance (Stankovic, MS, 2011).

Results and Discussion

a. **Results**

1) Flavonoid Compounds Qualitative Test

Table.1 Qualitative test results reagent with FeCl3

Sample	Reagent	Picture	Description	Results

Ethanol Extract 70%	FeCl3		Green bluish	+++
Ethanol Extract 96%	FeCl3		Green bluish	+++

Information:

a) *The positive symbol (+) indicates the reaction esults from the study are considered in accordance with the literature (Description).*

b) *The negative symbol (-) indicates the reaction results from the study are not in accordance with the literature (Description).*

Table 2. Qualitative test results reagent with HCL .

Sample	Reagent	Picture	Description	Results
Ethanol Extract 70%	HCL concentrated		Brownish yellow	+++
Ethanol Extract 96%	HCL concentrated		Brownish yellow	+

Information:

a) *The positive symbol (+) indicates the reaction esults from the study are considered in accordance with the literature (Description).*

b) *The negative symbol (-) indicates the reaction results from the study are not in accordance with the literature (Description).*

Table 3. Results of qualitative test using concentrated Ammonia reagent KLT method

Mobile Phase	Sample	Reagent	No UV Rays	With UV Rays	Results
(BAA) (4:1:5)	Ethanol : Extract 70% : 96%	FeCl3			-

Table 4. Results of the qualitative test using the Sitoborate reagent TLC method

Mobile Phase	Sample	Reagent	No UV Rays	With UV Rays	Results
(BAA) (4:1:5)	Ethanol : Extract 70% : 96%	Sitoborat			-

Table 5. The results of the qualitative test using the Sitoborate reagent TLC method

Mobile Phase	Sample	Reagent	No UV Rays	With UV Rays	Result
(BAA) (4:1:5)	Ethanol : Extract 70% : 96%	Sitoborate			-

Information:

a) *The positive symbol (+) indicates the reaction esults from the study are considered in accordance with the literature (Description).*

b) *The negative symbol (-) indicates the reaction results from the study are not in accordance with the literature (Description).*

2) Quantitative Test.

a) Quercetine Maximum Wavelength

The determination of the maximum wavelength of quercetin was carried out running and the results were obtained, namely the 70% ethanol extract obtained a wavelength of 429nm while the 96% ethanol extract obtained a wavelength of 426nm.

b) Operating Time

Table 6. Results Data Operating time Quercetin Ethanol 70% (429nm)

Time (minutes)	Average result
5	1,879
10	1,814
15	1,744
20	1,640
30	1,639

From these data, it was found that the operating time was 30 minutes marked by stable absorbance data or not much different at 30 minutes from the previous data.

Table 7. Results Data Operating time Quercetin Ethanol 96% (426nm)

Time (minutes)	Average result
5	2,751
10	2,723
15	2,711
20	2,702
30	2,701

From these data, it was found that the operating time was at 30 minutes marked by stable absorbance data or not much different at 30 minutes from the previous data.

c) Quercetin Wavelength

Table 8. Wavelength Quercetin Ethanol 70% (429nm)

Concentration	Absorbance (y)			Average
6	0.315	0.313	0.314	0.314
8	0.364	0.365	0.365	0.365
10	0.463	0.464	0.464	0.464
12	0.609	0.607	0.607	0.608
14	0.702	0.703	0.703	0.703

Table 9. Wavelength Quercetin Ethanol 96% (426nm)

Concentration	Absorbance (y)			Average
6	0.629	0.631	0.631	0.630
8	0.895	0.896	0.896	0.896
10	0.977	0.974	0.975	0.975
12	1.093	1.095	1.097	1.095
14	1.317	1.321	1.323	1,320

d) Standard Curvea

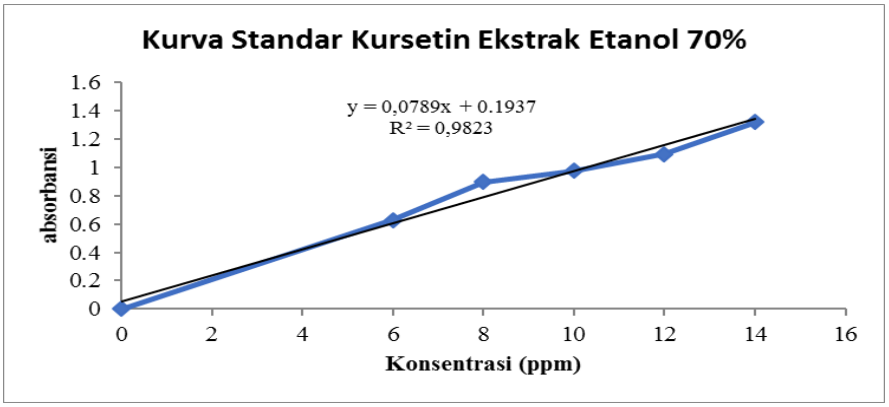

Table 10. Total Flavonoid Content Ethanol Extract 70%

Extract Weight(gram)	Average Absorbance (ɣ)	Total Flavonoid Content (%)
0.015	0.469	2,138

Table 11 Total Flavonoid Content Ethanol Extract 96%

Extract Weight (grams)	Average Absorbance (ɣ)	Total Flavonoid Content (%)
0.015	0.426	0.109

Discussion

In this study, a solvent solution, namely ethanol, was used to determine the content of flavonoids in which ethanol is polar. The nature of the polarity of the filter dramatically affects the extraction of compounds from the raw material. Flavonoid compounds are generally in the form of polar glycosides, so they must be dissolved in polar solvents (Yusoff et al., 2015).

The ethanol solvents used were 70% ethanol and 96% ethanol, the choice of concentrations of 70% ethanol and 96% ethanol because ethanol solvents with these concentrations were the easiest to find and the most commonly used. The purpose of the concentration comparison test was to find the most effective concentration of ethanol solvent for extracting the simplicia of mahogany seeds, making it easier for further research in the management of simplicia of mahogany seeds. This research was carried out with several stages of work procedures, namely sampling, simplicia management, extract making, qualitative and quantitative tests.

A sampling of mahogany seeds was carried out in the Tamiang Layang area, Central Kalimantan. The fruit taken is a fruit that has ripened and falls by itself from the tree because empirically or from generation to generation, the fruit that can be processed into traditional medicine is the fruit that has fallen from the tree. Mahogany seeds must be taken in the same area and from trees not too far apart to compare flavonoid content effectively.

The simplicia management of mahogany seeds is carried out in several stages, namely wet sorting, washing, drying, smoothing mahogany seeds, dry sorting and storage. Wet sorting is done by sorting mahogany seeds from foreign materials that are not useful or harmful at the time of making simplicia, washing mahogany seeds is done to reduce microorganisms that stick to the material and remove dirt, drying on simplicia is useful to reduce the content water content, the drying of mahogany seeds is carried out for about 2 weeks and directly under the sun because the seeds are quite thick and contain a lot of water, then the mahogany seeds are mashed using a blender to facilitate the extraction process because the more surface area of the sample, the faster the extraction process. . Before packaging simplicia, simplicia must be sorted dry first to separate foreign objects

that are still left behind and then stored in a dry, not humid and protected from direct sunlight that can damage the content contained in simplicia and avoid fungal contamination.

Extraction in this study uses the maceration method or the simplest method, the maceration method is carried out without a heating process with the principle of binding active substances based on their solubility properties, where the cell contents will be dissolved due to the difference in concentration between the solution inside the cell and outside the cell. A solution with a higher concentration will be pushed out and replaced by a liquid with a lower concentration, this process is called the diffusion process (Afifah, 2012).

The manufacture of simplicia extract of mahogany seeds was carried out using two types of ethanol solvents to determine the comparison, then the extraction stage was carried out with the same treatment. Several things that must be considered in processing this extract, namely the amount of simplicia, the amount of solvent used, the stirring time must be the same between 70% ethanol extract and 96% ethanol extract. After extracting the simplicia, the liquid extract was thickened on a water bath, in this study the thick extract process was carried out for 10 hours and the temperature must always be measured.

The temperature in the extract thickening does not exceed 60℃ so that the flavonoid compounds contained in the extract are not damaged. The higher the temperature in the extraction process, the flavonoid content will also decrease, this can happen because flavonoids are easily damaged at high temperatures. The decrease in flavonoid content can occur because high temperatures can damage the cell structure of the material so that the existing components easily migrate and become easily damaged by various chemical reactions that include light and oxygen (Sa'adah et al, 2017).

Analysis of flavonoid content was carried out to see whether this mahogany seed extract contained flavonoids. Several reagents were used to see the flavonoid content such as FeCl3 and concentrated HCL reagents. In this study, the extract was tested using these two reagents and the results showed that the mahogany seed extract contained flavonoids. To ensure that the color in the test tube does

show flavo-noid content, another TLC test or thin layer chromatography was carried out by spraying the reagent on the TLC plate, but in this study there was no color change on the TLC plate. Some of the factors that resulted in no color change were the wrong way of spraying the reagent or the color of the extract that was too thin so it was difficult to see the color change that occurred.

The results of the absorbance measurement used a concentration series of 6 ppm, 8 ppm, 10 ppm, 12 ppm and 14 ppm. Absorbance measurements were carried out using UV-Vis spectrophotometry. In 70% ethanol, the wavelength obtained is 429 nm. The absorbance values for 70% ethanol were 6ppm (0.630), 8ppm (0.896), 10ppm (0.975), 12ppm (1.095) and 14ppm (1.320). While in 96% ethanol the wavelength is 426 nm and the absorbance values are 6ppm (0.314), 8ppm (0.365), 10ppm (0.464), 12ppm (0.608) and 14ppm (0.703). The results of each solvent show the higher the concentration of the standard curve measurement, the higher the absorbance value as well. This calculation is in accordance with the Lambert-Beer law which shows that there is a straight (linear) relationship between the analyte content and its absorbance.

Using a concentration series because this method uses a standard curve equation, to make a standard curve, several concentration series are made to obtain a linear regression equation that can be used to calculate the percent concentration. In this study, quercetin was used as a standard solution because quercetin is the most widely distributed compound found in plants (Chang dkk, 2002).

The average results obtained are then entered into a linear equation with the formula ($y = bx + a$) obtained for each extract, namely 70% ethanol $y = 0.0789.x + 0.1937$ and the correlation coefficient is ($r= 0.9823$), while in ethanol 96% $y= 0.051.x + 0.0197$ and obtained a correlation coefficient of ($r= 0.9890$). If the value (r) is close to 1, it shows that the level of confidence is very strong and the curve formed is linear. The sample used was 0.0015gr / 15mg of mahogany seed extract. The total flavonoid content of each mahogany seed sample was obtained at 70% ethanol 2.138% while in ethanol 96% 0.109% where the total flavonoid content in 70% ethanol was higher, the higher the concentration of ethanol, the lower the

polarity level. from that 70% ethanol is more polar than 96% ethanol, so it is more effective in extracting the extract.

According to the Directorate General of POM (2014) the range of total flavonoid content sees the value of the absorbance ranges from (0.2-0.8). The absorbance values obtained in the 70% ethanol extract were 0.470, 0.468, 0.471, respectively. While the ethanol 96% 0.426, 0.427, 0.427. The results of this study have shown that the samples of 70% ethanol extract and 96% mahagony seeds contain flavonoid compounds.

Pharmacologically, mahogany seed extract contains flavonoid compounds which are proven to have antioxidant activity as a treatment for hypertension, diabetes mellitus and others (Roni and Hanny 2016). From the results of the research that has been done, it can be used as a reference in the selection of solvents to extract mahogany seeds that 70% ethanol is more effective.

Conclusion

Based on the results of the research that has been done, it can be concluded that 70% and 96% ethanol extracts contain flavonoid secondary metabolites. The results of the measurement of total flavonoid levels, concluded that 70% solvent was more effective in extracting flavonoid compounds.

Acknowledgements

The researcher would like to thank herself and all parties involved in this research. The researchers also express their gratitude to the Chemistry Laboratory of Sari Mulia University Banjarmasin which has provided facilities to support the implementation of this research.

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