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Comparison Method of Extraction to Total Flavonoid Convention of 70% Ethanol Extract of Gaharu Leaves (*Aquilaria microcarpa* Baill)

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ABSTRACT

Gaharu (Aquilaria microcarpa Baill) is one of the most common plants found in Sumatra and Kalimantan. Gaharu leaves (A. microcarpa Baill) contain flavonoid compounds that are useful as antioxidants. The purpose of this study was to compare the extraction method on the total flavonoid content of 70% ethanol extract of Gaharu (A. microcarpa Baill) leaves. Gaharu leaves were extracted through two extraction methods, namely percolation and soxhletation with 70% ethanol as solvent. Extracts based on each extraction method were tested for flavonoid qualitatively using Wilstater Test reagent by adding concentrated Mg and HCl. The flavonoid test was carried out quantitatively by colorimetric method using AlCl3 reagent and acetic acid with comparison of quercetin using UV-Vis spectrophotometry to measure total flavonoid content. The wavelength used in the determination of flavonoid content is 410 nm using an operating time of 40-60 minutes. The results of the identification of flavonoid compounds obtained from 70% ethanol extract of Gaharu leaves were positive for flavonoid compounds. The results of the quantitative test showed that the flavonoid content of the percolation method was 109.923 mg QE/g extract and the results of the soxhletation method was 36,333 mg QE/g extract. Based on these results indicate that the percolation method has higher levels of flavonoids than the soxhletation method.

Keywords**:** *Flavonoid Levels, Gaharu Leaves (A. microcarpa Baill), Percolation, Sokletation, UV-Vis Spectrophotometry.*

INTRODUCTION

Indonesia is a tropical country that has a very diverse biological wealth. This is in line with the number of people who have turned to traditional medicine by using medicinal plants to maintain health and also treat a disease (Sari et al., 2018). The increase in the use of herbal medicines is due to the slogan back to nature. This is in line with the many developments in research on plants or plants into drugs such as antioxidants, antivirals, anti-inflammatory, anti- allergic, anticancer. The pharmacological effects of medicinal plants are thought to be related to the important role of secondary metabolites, one of which is flavonoids (Gunawan et al., 2016).

Flavonoids are the largest class of natural phenolic compounds found in plants. Flavonoid compounds are found in almost all parts of plants including fruit, roots, leaves, and outer bark of stems (Worotikan, 2011). A number of plants and medicinal plants containing flavonoids have been reported to have antioxidant, antibacterial, antiviral, anti-inflammatory, anti-allergic, and anticancer activities (Ahmad et al., 2015). Therefore, it is important to know the flavonoid content of each plant. One of the plants that contain flavonoid compounds is Gaharu leaves (*A. microcarpa* Baill) (Fauzi et al., 2017).

Gaharu (*A. microcarpa* Baill) is a plant that is commonly found in Sumatra and Kalimantan. Several studies have reported that *A. microcarpa* Baill leaves have a pharmacological effect due to the content of chemical compounds from the flavonoid group that are used by the community as brewed drinks (Silaban, 2014). Amalina's research (2015) stated that the 70% ethanol extract of Gaharu (*A. microcarpa* Baill) leaves contains flavonoid

compounds, tannins & phenols. The content of flavonoid compounds in Gaharu (*A. microcarpa* baill) leaves is thought to play an important role in its activity as an antioxidant, which is shown to be in the very strong category (IC50 39.28 ppm). Given the importance of flavonoid compounds in pharmacological activity, it is necessary to conduct research on total flavonoid levels in the extract of Gaharu (*A. microcarpa* Baill) leaves. Researchers are interested in knowing the comparison of the extraction method on the flavonoid content of 70% ethanol extract of Gaharu (*A. microcarpa* Baill) leaves. The extraction method in this research is the cold extraction method, namely percolation and the hot method, namely soxhletation.

RESEARCH METHODOLOGY

The tools

The tools used in this study were glassware, stirring rod, evaporating dish, watch glass, cuvette, round bottom flask (Duran®), micropipette (Dragon Lab®), water bath, percolator, dropper pipette, rotary evaporator, spatula, UV-Vis spectrophotometer (PG Instrument®), stopwatch, test tube (Pyrex®), analytical balance (Ohaus®), and vial.

The materials

The materials used in this study were Aluminum Chloride ($AlCl_3$) (Merck®), Amyl Alcohol (Merck®), Acetic Acid, Hydrochloric Acid (HCl) (Merck®), Aquadest, Gaharu Leaves (*Aquilaria microcarpa* Baill), Ethanol 70 % (Onemed®), Ethanol pro analitic, Quercetin (Sigma aldrich®), and Magnesium (Mg) (Merck®).

Extract Making

The percolation method was carried out by weighing 200 grams of simplicia, put into a percolator vessel, and added 70% ethanol solvent to taste until Gaharu leaf (*A. microcarpa* Baill) simplicia was submerged and there was a liquid filter on it, the percolator was closed using aluminum foil and left for 24 hours. Then the percolator faucet was opened and the extract liquid was allowed to drip at a rate of 20 drops per minute and 70% ethanol solvent was added through the reservoir tube and the rate of dripping was adjusted to the speed of the percolate droplets so that there should always be a layer of solvent on top of the simplicia. Percolation was stopped when the last 1 ml of percolate came out. Separation of the extract from the solvent was carried out using a rotary evaporator with low pressure at a temperature of more than 50ºC. Then the extract was concentrated by evaporating it over a water bath with the lowest possible temperature, which was around 60ºC until a constant weight was obtained.

The soxhletation method was carried out by weighing 50 grams of simplicia. Wrapped using filter paper and tied with thread, inserted into the Soxhlet apparatus. 250 mL of 70% ethanol was added to the flask then soxhletation was carried out at a temperature of 60^{o}C-70^{o}C until a colorless or faded cycle was obtained, then the extract was separated from the solvent using a rotary evaporator at a temperature of 40^{o}C. The extract was concentrated using a water bath at a temperature of 40^{o}C until it reached a constant weight.

Flavonoid Identification

The flavonoids were identified by weighing 0.5 g of extract added with 10 ml of distilled water, then adding 0.1 g of concentrated Mg powder and 1 ml of concentrated HCl and 1 ml of amylalcohol then shaking vigorously, forming a red, yellow, or orange solution on the surface. Amylalcohol layer which indicates the presence of flavonoid compounds.

Determination of Flavonoid Level

Determination of Quercetin Maximum Wavelength

Take 1 mL of 500 ppm quercetin solution added with 1 mL of 10% AlCl3 and 8 mL of 5% acetic acid. It was allowed to stand for 30 minutes and read using UV-Vis spectrophotometry with a wavelength of 350-450 mm.

Determination of the standard quercetin curve

Determination of the standard quercetin curve by taking a serial solution of quercetin dilution levels of 100, 200, 300, 400, and 500 ppm series concentration from each taken as much as 1 mL of the concentration series solution and each concentration is reacted with 1 mL of 10% AlCl3 and 8 mL of 5% acetic acid. Incubated according to the results of Operating Time and the absorbance readings were carried out using UV-Vis spectrophotometry with the maximum wavelength that had been obtained.

Determination of Flavonoid Levels Total

Determination of Flavonoid Levels Total sample of 70% ethanol extract of Gaharu (*A. microcarpa* Baill) leaves by percolation and soxhletation methods with a concentration of 1000 ppm each taken or pipette as much as 1 mL, added with 1 mL of 10% $AlCl_3$ and 8 mL of Acetic Acid 5 % were incubated according to the Operating Time results obtained.

Data Analysis

The concentration results data made from the quercetin standard were then formed by the standard curve equation. The standard curve equation is y = bx+a where y is the absorbance unit nm, x is the level in ppm (mg/L). The absorbance of 70% ethanol extract of Gaharu leaves was entered into the standard curve equation, then included in the formula for determining the flavonoid content

$$\text{Total Flavonoid Content} = \frac{C \; x \; V}{M}$$

Description : C = Quercetin Equivalence (mg/L)
V = Total volume of ethanol extract (mL)
M = Sample weight (mg)

RESULTS AND DISCUSSION

Dari dua ratus gram serbuk simplisia Daun Teratai yang disoxhletasi dengan 1 L pelarut didapatkan ekstrak kental dengan berat 8,19 g dengan persen rendemen 4,09%. Uji Kualitatif dilakukan dengan menguji adanya kandungan Flavonoid, Tanin, Polifenol, Alkaloid, dan Saponin. Hasil pengujian ini dapat dilihat pada Tabel 1.

Table 1. Data on Rendemen of 70% Ethanol Extract of Gaharu Leaves (*A. microcarpa* Baill) by Percolation and Soxhletation Methods

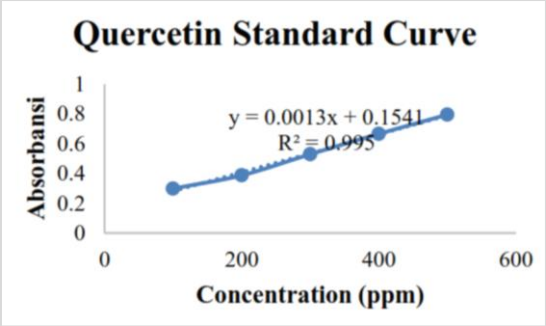
Ingredients	Powder Weight (g)	Extract (g)	Rendemen (%)
Extract by Percolation Method	200	43,171	21,5855
Extract by Soxhletation Method	50	11,454	22,9082

Table 2. Table 2. Results of Identification of Flavonoids Ethanol Extract 70% Gaharu Leaves (*A. microcarpa* Baill)

Method	Documentation	Result	Description
Percolation		+	A yellow solution is formed on the amyl alcohol layer
Soxhletation		+	An orange solution is formed on the amyl alcohol layer

Description: (+) = contains the test compound; (-) = does not contain the test compound.

The maximum wavelength of quarcetin is 410. The results of the determination of the absorbance operating time in 60 minutes with an interval of every 5 minutes obtained a stable absorbance value at 40-60 minutes. Then the standard curve for quercetin was determined by making 1000 ppm mother liquor. Furthermore, serial solutions with levels of 100, 200, 300, 400, and 500 ppm were made. For each series of solution levels, absorbance readings were carried out with the maximum wavelength obtained, namely 410 nm using a UV-Vis spectrophotometer. The results of the linear regression equation obtained are y = 0.0013x + 0.1541 with a correlation coefficient (r) of 0.995. The standard curve of quercetin can be seen in Figure 1.



Picture 1. Quercetin Standard Curve

Determination of total flavonoid content of 70% ethanol extract of Gaharu (*Aquilaria microcarpa* Baill) leaves made with a concentration of 1000 ppm (10mg/10mL) and 3 times replication. Samples of 70% ethanol extract of Gaharu leaves were read for absorbance with a maximum wavelength of 410 nm using a UV-Vis spectrophotometer. The results of the determination of flavonoid levels can be seen in Tables 3 and 4.

Table 3. Total Flavonoid Content Ethanol Extract 70% Gaharu Leaf Percolation Method

Sample	Sample absorbance	mg QE / g Extract	mg QE / g Extract ± SD
Percolation Extract	0,295	108,3846	109,923 ± 1,53845
	0,297	109,9231	
	0,299	111,4615	

Table 4. Total Flavonoid Content Ethanol Extract 70% Gaharu Leaf Soxhletation Method

Sample	Sample absorbance	mg QE / g Extract	mg QE / g Extract ± SD
Soxhletation Extract	0,202	36,8462	36,33337 ± 0,88825
	0,200	35,3077	
	0,202	36,8462	

The average yield obtained from the 70% ethanol extract of Gaharu leaves with the percolation method was 109.923 mg QE/g extract and the soxhlet method was 36,33337 mg QE/g extract.

Based on the results of the determination of flavonoid levels, the percolation method was greater than the soxhletation method. The difference in these results may be caused by heating in the soxhletation method, thereby affecting the decrease in flavonoid content (Rahman et al., 2017). In the opinion of Sa'adah et al., (2017) stated that the heating process can cause a reduction in total flavonoid levels by 15-78%. There is a relationship between temperature and phenolic content, i.e. increasing temperature results in an increase in phenolic content up to a certain temperature and then decreases along with an increase in higher temperature. Flavonoids are phenolic compounds that have a conjugated aromatic system. The conjugated aromatic system can be easily damaged at high temperatures. Several groups of flavonoids have glycosidic bonds with sugar molecules. Glycoside bonds can be easily broken

and broken at high temperatures. In terms of active substances, in general, flavonoids are thermostable compounds, but there are also flavonoids that have thermolabile properties (Rahman et al., 2017).

CONCLUSION

Based on the results of the study, it can be concluded that the flavonoid content of 70% ethanol extract of Gaharu (*A. microcarpa* Baill) leaves using the percolation method obtained an average of 109.923 mg QE/g extract with a percentage of 10.993 (w%/w) and the soxhlet method obtained an average 36.3333 mg QE/g extract with a percentage of 3.6333 (w%/w).

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