Original Article

p-ISSN : 2442-6032
e-ISSN : 2598-9979

DOI : 10.35311/jmpi.v9i2.360

Phytochemical Screening Analysis and Determination of Total Flavonoids and Total Phenolics Content of Ethanol Extract of Sungkai Leaf (*Penorema canescens* Jack) from Samarinda City

Muhammad Ma'ruf*, Moch. Saiful Bachri, Laela Hayu Nurani

Faculty of Pharmacy, Universitas Ahmad Dahlan Yogyakarta, 55164, Indonesia

Citation: Ma'ruf, M., Bachri, M. S., & Nurani, L. H. (2023). Phytochemical Screening Analysis and Determination of Total Flavonoids and Total Phenolics Content of Ethanol Extract of Sungkai Leaf (*Penorema canescens* Jack) from Samarinda City. *Jurnal Mandala Pharmacon Indonesia*, 9(2), 262-272. https://doi.org/10.35311/jmpi.v9i2.360

Submitted: 16 July 2023
Accepted: 12 October 2023
Published: 23 December 2023

*Corresponding Author:
Muhammad Ma'ruf
Email: muhammadmaruf2400@gmail.com

ABSTRACT

Sungkai is a plant that contains compounds such as flavonoids, tannins, phenolics, saponins, steroids, and terpenoids, which have the potential to develop native Indonesian herbal medicines to treat the prevention and treatment of various diseases. This research aimed to analyze phytochemical screening and the total flavonoid and phenolic content in sungkai leaves originating from Samarinda City. This research method was carried out by extracting sungkai leaves with 96% ethanol and evaporating them with a vacuum rotary evaporator. Sungkai leaf extract was analyzed for phytochemical screening using the tube method with color testing from various reagents and total phenolic and flavonoid content testing using the colorimetric method using a UV-Vis spectrophotometer. The results of the phytochemical screening showed that the ethanol extract of sungkai leaves from Samarinda City was positive for containing flavonoids, saponins, alkaloids, phenolics, and steroids. The results of determining the flavonoid and phenolic content of sungkai leaf ethanol extract were 18,210 ± 0.271 mg QE/g sample and 33,666 ± 1.052 mg GAE/g sample.

Keywords: Sungkai, Screening, Phytochemical, Flavonoid, Phenolic

ABSTRAK

Sungkai merupakan salah satu tumbuhan yang memiliki kandungan senyawa seperti flavonoid, tanin, fenolik, saponin, steroid, dan terpenoid yang memiliki potensi sebagai pengembangan obat herbal asli Indonesia untuk menangani pencegahan dan pengobatan dari berbagai penyakit. Tujuan penelitian ini adalah untuk menganalisis skrining fitokimia serta analisis kandungan flavonoid dan fenolik total pada daun sungkai yang berasal dari Kota Samarinda. Metode penelitian ini dilakukan dengan mengekstrak daun sungkai dengan etanol 96% dan diuapkan dengan *vaccum rotary evaporator*. Ekstrak daun sungkai dianalisis skrining fitokimia menggunakan metode tabung dengan uji warna dari berbagai pereaksi dan uji kandungan fenolik total dan flavonoid total dengan metode kolorimetri menggunakan spektrofotometer UV-Vis. Hasil dari skrining fitokimia diperoleh bahwa ekstrak etanol daun sungkai yang berasal dari Kota Samarinda positif mengandung flavonoid, saponin, alkaloid, fenolik, dan steroid. Dan hasil penetapan kadar flavonoid total serta fenolik total ekstrak etanol daun sungkai berturut-turut sebesar 18,210 ± 0,271 mg QE/g sampel dan 33,666 ± 1,052 mg GAE/g sampel.

Kata Kunci: Sungkai, Skrining, Fitokimia, Flavonoid, Fenolik

INTRODUCTION

Indonesia is an archipelagic country which has various kinds of natural resources. One of the wealth owned by Indonesia is its flora and fauna. The wealth of flora owned by Indonesia is included in the category of medicinal plants. Indonesia has about 30,000 types of plants and 7,000 other types of plants that can be used as medicinal plants (Jumiarni & Komalasari, 2017). One example of a plant that can be used as traditional medicine and whose efficacy has not been scientifically proven is sungkai (*Peronema canescens* Jack).

According to Adlis (2020), Sungkai leaves obtained from Padang Pariaman Regency with hexane, ethyl acetate, and

ethanol extracts of Sungkai leaves contain secondary metabolites,namely flavonoids, phenolics, saponins, steroids, and alkaloids. Plants that contain flavonoids can inhibit the activity of angiotensin-converting enzymes, reduce oxidative stress, and increase the relaxation of the blood vessel endothelium, which decreases blood pressure (Widiasari, 2018). A study conducted by Carolina et al., (2022), to giving sungkai leaf infusion to older adults in the Pahandut Palangka Raya Health Center area resulted in changes in blood pressure that reduced blood pressure in hypertensive older adults. So that Sungkai leaves can be efficacious in the treatment of hypertension.

Phytochemical screening is one way to identify a plant's secondary metabolite content. Phytochemical screening using the tube method was chosen because phytochemicals can determine the properties of active substances that cause toxic or beneficial effects on crude extracts if tested in a biological system (Shofi et al., 2020). Therefore, it is necessary to carry out further research on the ethanol extract of Sungkai leaves from Palaran District, Samarinda City, East Kalimantan, to determine the secondary metabolites contained in the form of flavonoids, saponins, alkaloids, phenolics, and steroids and to analysis of the total flavonoid and total phenolic content

METHOD

Tools

The tools used in this research were a vacuum rotary evaporator (Heidolph), glassware (Iwaki), analytical scales (Ohaus), micropipette (Socorex), UV-VIS spectrophotometer (Shimadzu UV-1800).

Materials

The materials used in this study were sungkai leaves (*Peronema canescens* Jack) from Palaran District, Samarinda City, East Kalimantan, Dragendroff peraction, Mayer peraction, solution of iron (III) chloride solution of 10%, ethanol 96%, quercetin, gallic acid, NaOH, and $AlCl_3$.

Identification of Sungkai Plants

The sample used in this study was carried out. Identification of plants was carried out to establish the truth of the material under investigation. Identification of Sungkai leaf plants is carried out at the UPT. Herbal Materia Medica Laboratory, Batu City, East Java, with letter number: 067/1382/102.20/2023. Sungkai leaves used are the older parts of the leaves and get enough sunlight. After washing with running water, the leaves are dried and protected from direct sunlight. The signs that the sungkai leaves are ready to be mashed have shown that if they are squeezed, they will break easily. Furthermore, sungkai leaves were mashed and sieved using sieve number 60.

Sample Preparation of Sungkai Leaf Ethanol Extract

The preparation of this extract follows the method used by Sadik (2021), macerating 1 kg of dried Sungkai leaf simplicia with 96% ethanol solvent with a ratio of 1:5 for 3 days. Then, the maceration results were evaporated using a vacuum rotary evaporator to obtain a thick ethanol extract of Sungkai leaves.

Phytochemical Screening Analysis

In the phytochemical screening analysis of the ethanol extract of sungkai leaves, tests were carried out, which included reaction tests on color changes and reaction tests on precipitation as follows:

1. Flavonoid Test

The Flavonoid test was carried out with Mg and 2N HCl reagents. A positive test for flavonoids is indicated by reddish-black, yellow, or orange precipitates (Febri Fatwami et al., 2023).

2. Saponin Test

The Saponin test was carried out with hot distilled water and 2N HCl. Stable foam indicates a positive saponin test, and the foam does not disappear when added with HCl 2 N (Larasati & Putri, 2023).

3. Alkaloid Test

The alkaloid test was carried out with Dragendorf reagent. A positive test for alkaloids is indicated by the presence of an

orange-to-yellow precipitate (Imran et al., 2023).

4. Phenolic Test

The phenolic test was carried out with a $FeCl_3$ reagent. A positive test for phenolic is indicated by the formation of a blue or dark blue-green color (Rifkia & Prabowo, 2020).

5. Steroid Test

The steroid test was carried out by reacting anhydrous acetic and sulfuric acid. A positive steroid test is indicated by the formation of a bluish-green color (Silvani et al., 2023).

Determination of Total Flavonoid Content by UV-Vis Spectrophotometry

In conducting a quantitative analysis of the total flavonoid content, follow the method from Ramadhani et al., (2022) with a few modifications. The operating time was determined with quercetin mother liquor for 60 minutes at a theoretical maximum wavelength of 415 nm. Then, the maximum wavelength of quercetin was determined and incubated according to the operating time obtained and then read at a wavelength of 350-500 nm. Comparison solutions were prepared with concentrations of 125, 100, 75, 50, and 25 µg/ml incubated according to the operating time and measured at the maximum wavelength according to the results of the previous measurements. Weigh 0.1 gram of ethanol extract from Sungkai leaves and then dissolve it with ethanol until the volume is 10 mL. Take 0.5 mL of the previous solution, then add 1.5 ml of ethanol, 0.1 mL of 10% AlCl3, 0.1 ml of 1M sodium acetate, and 2.8 ml of distilled water. Then, it was incubated according to the operating time, and the absorbance was measured at the maximum wavelength of the previous measurement results. The sample absorbance measurement procedure was repeated 5 times to minimize errors, and the results obtained were expressed in mg QE/g simplicia.

Determination of Total Phenolic Content by UV-Vis Spectrophotometry

In conducting a quantitative analysis of total phenolic content, follow the method from Ramadhani et al., (2022) with a few modifications. The operating time was determined with gallic acid mother liquor and then read at a wavelength of 730 nm every minute until the 90th minute. Then, the gallic acid wavelength was determined. Maximum wavelength measurements were made using visible spectrophotometry at 550-800 nm. Then, a series of concentration solutions were created with concentrations of 30, 40, 50, 60, 70, and 80 µg/ml and incubated according to the operating time that had been obtained. The absorbance was measured at the maximum wavelength that had been obtained previously. Weigh 0.2 grams of ethanol extract from Sungkai leaves and dissolve it with methanol p.a until the volume is 25 mL. Take 1 mL of the once-diluted solution and add 5 mL of Folin-Ciocalteu solution. And they added 4.0 mL of NaOH. Then, it was incubated according to the operating time, and the absorbance was measured at the maximum wavelength of the previous measurement results. The sample absorbance measurement procedure was repeated 5 times to minimize errors, and the results obtained were expressed in mg QE/g simplicia.

RESULTS AND DISCUSSION

Extraction of Sungkai Leaf Ethanol Extract

The yield of macerate obtained from the evaporation of the solvent using a vacuum rotary evaporator is 125 grams of thick extract. A vacuum rotary evaporator is used because it has the principle of evaporating solvent below its boiling point by lowering the pressure using a vacuum. The lower the pressure given, the faster the evaporation of the solvent obtained so that the sungkai viscous extract obtained was as much as 125 grams compared to the weight of the simplicial powder obtained.

$$\% \text{ yield} = \frac{\text{weight of viscous extract obtained}}{\text{weight of simplicia}} \times 100\%$$

$$= \frac{125 \text{ grams}}{1000 \text{ grams}} \times 100\%$$

$$= 12{,}5\%$$

The percentage yield of the ethanol extract of Sungkai leaves obtained was higher than that obtained in a study conducted by (Fadlilaturrahmah et al., 2021), which was 7.28%. This happens because it can be influenced by the location where the Sungkai plant grows. The percentage yield calculation is carried out to compare the final product obtained from the extraction process to the raw materials used before the extraction process so that the amount of raw materials needed to get the desired extract can be determined. The higher the yield percentage obtained, the more dissolved substances during the extraction process.

Phytochemical Screening Analysis

The results of the phytochemical screening analysis of the ethanol extract of Sungkai leaves (*Peronema canescens* Jack) originating from Samarinda City, East Kalimantan, can be seen in Table 1:

Table 1. Phytochemical Screening Analysis of Sungkai Leaf Ethanol Extract

No.	Test	Reactor	Results	Observation
1	Flavonoid	Mg dan HCl 2N	+	Yellow color formed
2	Saponin	Hot aquades and HCl 2N	+	Stable foam formed for 10 minutes and with the addition of 2N HCl the foam did not disappear
3	Alkaloid	Dragendorf	+	An orange precipitate formed
4	Fenolik	$FeCl_3$	+	Formed a bluish-green color
5	Steroid	Acetic anhydrous acid and sulfuric acid	+	Formed a bluish-green color

Phytochemical testing on Sungkai leaf extract consisted of flavonoids, saponins, alkaloids, phenols, and steroids. The results of the phytochemical screening showed that the ethanol extract of Sungkai leaves from Palaran District, Samarinda City, East Kalimantan, positively contained flavonoids, saponins, alkaloids, phenols, and steroids.

The ethanol extract of Sungkai leaves contains polar secondary metabolites. The content of phenolic and flavonoid compounds, which contain hydroxy groups, makes it easier for the polar solvent to attract these two compounds into the ethanol solvent, which is also rich in hydroxy groups (Ramadhani et al., 2022).

Based on the phytochemical tests conducted by (Rahman et al., 2022), it was found that Sungkai leaves originating from Merangin Regency, Jambi Province, also contain secondary metabolites of tannins, phenols, flavonoids, saponins, and alkaloids. Differences in the location where Sungkai grows can cause differences in the presence of secondary metabolites contained in the number of secondary metabolites contained in the Sungkai extract.

Determination of Total Flavonoid Content by UV-Vis Spectrophotometry

This research aimed to determine the total flavonoids content contained in the ethanol extract of Sungkai leaves using the UV-Vis Spectrophotometry method. The choice of this method is because it has the advantage that the process is quite simple, can be used to determine tiny quantities of substances, the results obtained are pretty fast and accurate, and the numbers that are read are recorded directly by the detector and printed in the form of digital numbers or graphs that have been regressed. The drawbacks of this method are that absorption is affected by the pH of the solution, temperature, the presence of interfering substances, and the cleanliness of the cuvette; it is used only in functional groups containing valence electrons with low excitation energy; the light used must be monochromatic (Rohmah et al., 2021).

Determination of the levels of flavonoids contained in the ethanol extract of Sungkai leaves was carried out using the UV-Vis spectrophotometry method because flavonoids have a conjugated chromophore group and an autochrome group that binds directly to the chromophore group (Susilowati & Sari, 2021). The use of the quercetin standard in the determination of flavonoid levels is because the quercetin compound has an amount of around 60-75% of the flavonoids. According to (Lindawati et al., 2020), the structure of quercetin is a flavonoid compound in the flavonol group with a keto group on C-4 and a hydroxyl group on C-3 and C-5 atoms. Quercetin is a flavonoid compound that can react with $AlCl_3$ and form complex reactions, which can be seen in Figure 1:

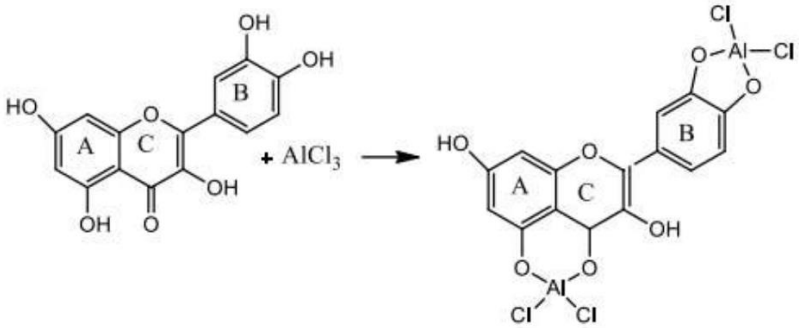

Figure 1. Formation of quercetin complexes with $AlCl_3$ reagent (Susilowati & Sari, 2021)

In determining the levels of flavonoids, a colorimetric reaction occurs because the sample reacts with $AlCl_3$ in acidic media. The addition of $AlCl_3$ will form a complex response between the flavonoids and $AlCl_3$ this causes a shift in the wavelength to the visible direction, producing a more yellow color (Lindawati et al., 2020) . Furthermore, adding sodium acetate in this experiment maintains the wavelength in the visible area (Asmorowati, 2019).

Operating time measurement is carried out before the measurement is carried out. This is because there is a possibility that the reaction of the complex compound that is formed could be better. Still, on the contrary, if the measurement is carried out after the operating time, there will be a possibility that the complex compound between $AlCl_3$ has been damaged (Suharyanto & Prima, 2020). This operating time measurement aims to determine the time when the quercetin uptake is stable (Damayanti et al., 2023). And obtained in the operating time measurement received a stable absorbance value at 28-32 minutes.

Determining the maximum wavelength helps determine the measurement wavelength of a complex compound between quercetin and $AlCl_3$ to provide optimal absorbance (Damayanti et al., 2023). The maximum wavelength in this study was obtained at 443.0 nm.

Determining the standard curve intends to recognize the relationship between the concentration of the solution and its absorbance value so that the attention of the test sample can be known (Hilma et al., 2021). The absorption results were read during the operating time for 30 minutes, with a maximum wavelength of 443.0 nm, which can be seen in Figure 2. The results of the measurements that have been carried out are the standard curve equation Y = 0.0037x + 0.2491 with an R-calculated value of 0.9932. From that day, according to the R table statistical test with the number of data (n) = 5 at the 95% confidence level, the value of the R table is 0.8783. The results show that the R count exceeds the R table. This indicates a solid significant relationship between the standard quercetin concentration and absorbance and the linear regression equation

(Asmorowati, 2019). With that, the normal curve of the linear regression equation that has been obtained is used to calculate the levels of flavonoids contained in the ethanol extract of Sungkai leaves.

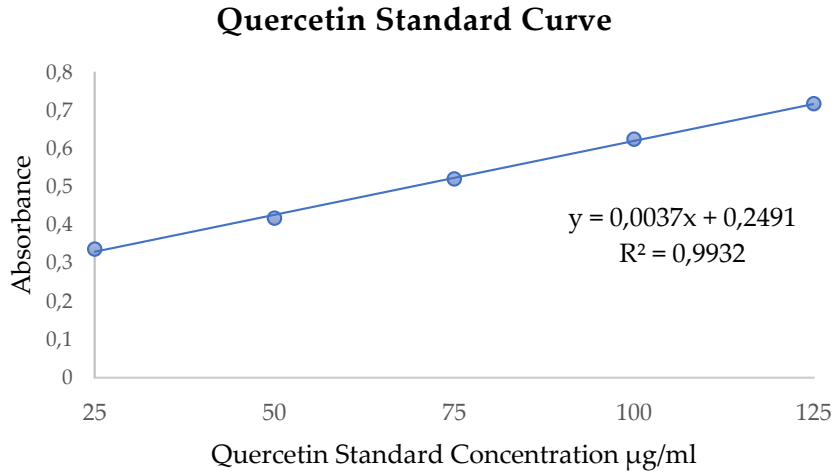

Figure 1. Standard curve graph on quercetin

In determining the levels of flavonoids expressed in QE (Quercetin Equivalent). QE is the equivalent number of milligrams of quercetin in 1 gram of sample (Hadi et al., 2023). The result of determining the total flavonoid content was 18.210 ± 0.271 mg QE/g, which means that every gram of ethanol extract of Sungkai leaves has the same value as 18.210 mg of quercetin and which can be seen in Table 2:

Table 2. Results of Total Flavonoid Content in Sungkai Leaf Ethanol Extract

Replication	Sample weight	Absorbance	Rate (mg QE/g)	Average	SD	CV
1	0,1004	0,592	18,461			
2	0,1002	0,581	17,905			
3	0,1003	0,587	18,210	18,210	0,271	1,486%
4	0,1005	0,593	18,497			
5	0,1001	0,582	17,977			

These calculations found that the CV value was 1.468%, which fulfilled the homogeneity requirements because the value was <5% (Sari & Fitrianingsih, 2020).
In a study by Adlis Santoni et al., (2023) on the hexane extract of Sungkai leaves originating from Padang City, West Sumatera, a total flavonoid content of 28.572 mgGE/g sample was obtained.

The Flavonoid content possessed by the ethanol extract of Sungkai leaves has oxidation-reducing properties, which have the potential as antioxidants. This is caused by the presence of hydroxy groups found in the structure of flavonoids. Flavonoids are indispensable in various biological uses, traditional pharmaceutical preparations, synthetic drugs, and cosmetics. This is attributed to its antioxidant, anti-inflammatory, antimutagenic, and anticarcinogenic properties coupled with its ability to regulate the body's metabolism (Panche et al., 2016).

Determination of Total Phenolic Content by UV-Vis Spectrophotometry

This research aimed to determine the total phenolic content contained in the ethanol extract of Sungkai leaves using the UV-Vis

Spectrophotometry method. This method is chosen because UV-Vis Spectrophotometry has the advantages of having a low detection limit and a high degree of accuracy and precision (Bahrum et al., 2023). The drawbacks of this method are that the compound to be analyzed must have a chromophore group (color carrier group), conjugated double bonds, and a wavelength that lies in the ultraviolet or visible region (Tetha & Sugiarso, 2016).

This research was conducted to determine the levels of total phenolic compounds in the ethanol extract of Sungkai leaves with the reference compound used, namely gallic acid solution, the reason for choosing gallic acid is because gallic acid is a derivative of hydroxybenzoate acid, which is classified as simple phenolic acid and the phenol content of gallic acid is pure (Tahir et al., 2017). In addition, gallic acid has a hydroxy group and conjugated double bonds in each benzene ring (Hilma et al., 2021), and the reaction can be seen in Figure 3:

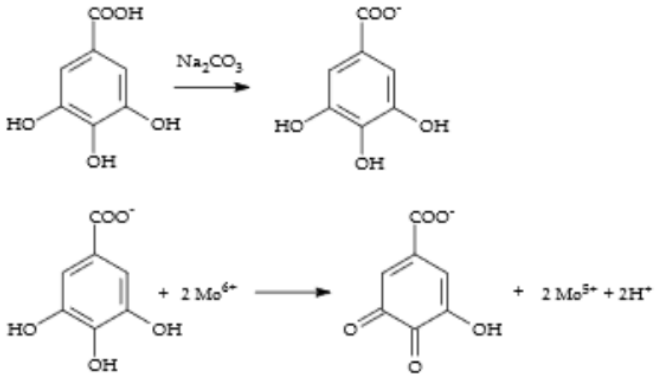

Figure 1. The reaction of gallic acid with the Folin-Ciocalteu reagent (Hilma et al., 2021)

The phenolic-hydroxy group reduces the heteropoly acid (phosphomolybdic phosphotungstate) contained in the Folin-Ciocalteu reagent into a blue molybdenum-tungsten complex which UV-Vis Spectrophotometry can detect phenolic compounds into phenolic compounds. Therefore, NaOH is used to make the atmosphere alkaline (Andriani & Murtisiwi, 2018).

The operating time on gallic acid was obtained at 57-61 minutes. In operating time, it is known that the absorption value is said to be stable if the absorbance value is the same or seen from the relatively small difference in absorbance value. With that in mind, the purpose of determining operating time is to determine the relationship between absorbance and time in the solution to be used (Riyanti & Wilianita, 2023).

The maximum absorption wavelength of gallic acid was obtained in the 61st minute after adding the Folin-ciocalteu reagent and NaOH using a UV-Vis spectrophotometer, and a blue solution was obtained. Then, absorption readings were taken in the range of 550-800 nm, and this absorption was used as the maximum wavelength. And the maximum wavelength obtained is 750.2 nm.

In determining the gallic acid standard curve, absorption readings were carried out during the operating time for 61 minutes and measured at a wavelength of 750.2 nm. The results of gallic acid absorbance can be seen in Figure 4. The results of the measurements that have been made are the standard curve equation Y = 0.0095x + 0.0507 with an R-calculated value of 0.9965. From that day, according to the R table statistical test with the number of data (n) = 5 at the 95% confidence level, the value of the R table is 0.8783. From the results obtained, it shows that the R count is greater than the R table. This indicates a solid significant relationship between the standard gallic acid concentration and the absorbance and the linear regression equation

(Asmorowati, 2019). With that, the standard curve of the linear regression equation that has been obtained is used to calculate the phenol content contained in the ethanol extract of Sungkai leaves.

In determining the total phenolic content of the ethanol extract of Sungkai leaves, measurements were made at 61 minutes and a wavelength of 750.2 nm. The absorption results read can be seen in Table 3:

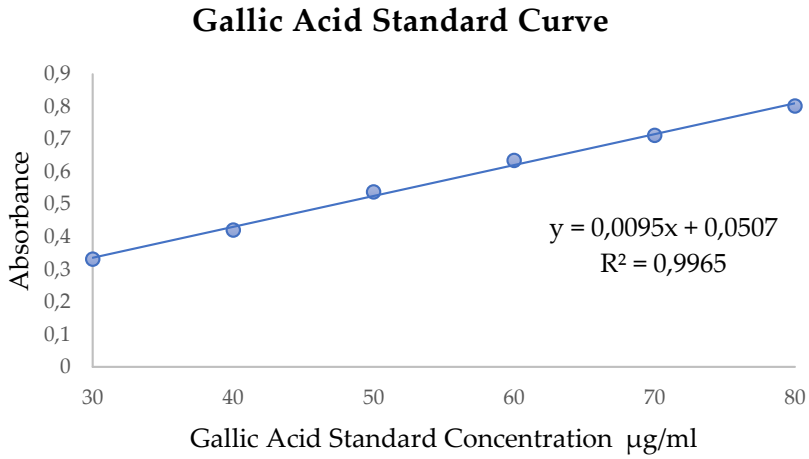

Figure 4. Standard curve graph for gallic acid

Table 3. Results of Total Phenolic Content in Sungkai Leaf Ethanol Extract

Replication	Sample weight	Absorbance	Rate (mg GAE/g)	Average	SD	CV
1	0,2003	0,582	34,902	33,666	1,052	3,126%
2	0,2001	0,551	32,898			
3	0,2002	0,567	33,933			
4	0,2004	0,573	34,293			
5	0,2001	0,542	32,306			

The result of determining the total phenolic content was 33.666 ± 1.052 mg GAE/g, which means that every gram of ethanol extract of Sungkai leaves has the same value as 33.666 mg of gallic acid. From these calculations, it was found that the CV value was 3.126%, where this value fulfilled the homogeneity requirements because the value was <5% (Sari & Fitrianingsih, 2020). In a study by (Adlis Santoni et al., 2023) on the hexane extract of Sungkai leaves originating from Padang City, West Sumatra, had a phenolic content of 3.582 mgGAE/g samples.

Several studies have shown that antioxidant activity is proportional to the content of phenolic compounds. Antioxidants are related to the ability to perform free anti-radical action from natural cell damage. Therefore, the flavonoids and phenols contained in Sungkai leaves are thought to function as antitumor, anti-inflammatory, and antimicrobial (Lubis et al., 2018).

Differences in the growing areas of the sample plants can cause the difference in total phenolic and flavonoid levels. This study used samples from Samarinda City, East Kalimantan, while a survey conducted by (Adlis Santoni et al., 2023) used samples from Padang City, West Sumatra. In addition, differences can be influenced by environmental factors such as rainfall,

temperature, ultraviolet radiation, and soil composition (Borges et al., 2013).

ACKNOWLEDGMENT

The authors are grateful to Departement of Education and Culture Office of East Kalimantan for providing a "Kaltim Tuntas" Scholarship 2023 to complete the Master of Pharmacy at Universitas Ahmad Dahlan.

CONCLUSION

It can be concluded from this study that the results of the phytochemical screening showed that the ethanol extract of Sungkai leaves originating from Samarinda City, East Kalimantan, positively contained flavonoids, saponins, alkaloids, phenols, and steroids. The results of determining the total flavonoids and phenolic content of the ethanol extract of Sungkai leaves were 18.210 ± 0.271 mg QE/g sample and 33.666 ± 1.052 mg GAE/g sample. Suggestions for future researchers, namely conducting activity tests on antioxidants from the ethanol extract of Sungkai leaves from Palaran District, Samarinda City, East Kalimantan.

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