DETERMINATION OF TOTAL FLAVONOID CONTENT OF 70% ETHANOL AND ETHYL ACETATE EXTRACT OF LAMTORO LEAVES (*Leucaena leucocephala* (Lam.) de Wit) USING UV-Vis SPECTROPHOTOMETRIC METHOD

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Abstract. Lamtoro leaf (*Leucaena leucocephala* (Lam.) de Wit) is one of the plants of the Fabaceae family. Lamtoro leaves have properties as antibacterial, antidiabetic, anti-inflammatory, anticancer, anthelmintic, and antioxidant. There are secondary metabolites such as flavonoids, alkaloids, tannins, and saponins. The purpose of this study was to determine the content of secondary metabolite compounds by phytochemical screening methods and determine the total flavonoid levels of lamtoro leaves *(Leucaena leucocephala* (Lam.) de Wit) with 70% ethanol solvent and ethyl acetate and find out whether or not there is a difference in total flavonoid levels of lamtoro leaves (*Leucaena leucocephala* (Lam.) de Wit). This research is an experimental study using the maceration method with 70% ethanol solvent and ethyl acetate, and a phytochemical screening test was carried out. The data obtained were analyzed using a normality test, a homogeneity test, followed by an independent T-Test. Lamtoro leaves *(Leucaena leucocephala* (Lam.) de wit) are positive for flavonoids, alkaloids, tannins, and saponins. The moisture content contained in simplisia powder is 4.55%. The flavonoid content of the 70% ethanol extract was 5.45% and the total flavonoid content of the ethyl acetate extract was 6.94%. Normality test of 70% ethanol extract and ethyl acetate of lamtoro leaves is normally distributed. The independent T-Test resulted in a difference between the total flavonoid levels of 70% ethanol extract and ethyl acetate extract of lamtoro leaves (*Leucaena leucocephala* (Lam.) de Wit) due to the sign results obtained, 0.002< 0.05.

Key words: [Lamtoro leaf (*Leucaena leucocephala* (Lam.) de Wit), ethyl acetate, 70% ethanol, UV-Vis spectrophotometry, total flavonoid levels]

INTRODUCTION

Indonesia is an archipelagic country with a vast territory. Medicinal plants are a crucial natural resource for treating and maintaining public health. One such herbal remedy is the lamotoro leaf (Sari et al., 2021). The lamtoro plant, also known as petai cina (Chinese stink bean), is a tropical plant abundant in several regions. This plant possesses potential bioactive compounds that can be used as herbal medicines (Praja & Oktarlina, 2017). The petai cina fruit is a legume containing numerous small seeds. The seeds are oval and flat, and when ripe, they turn brown and contain mimosine, leucanin, leucanol, and protein. *Leucaena leucocephala* (Lam.) is a member of the Fabaceae family. Pharmacologically, lamtoro has antibacterial, antidiabetic, anti-inflammatory, anticancer, anthelmintic, and antioxidant properties (Rivai, 2021). Leucaena contains bioactive compounds or secondary metabolites, including alkaloids, saponins, tannins, and flavonoids, which can be used as herbal medicines (Abriyani, 2018).

Flavonoids are secondary metabolites frequently found in nature. Flavonoids possess several colorants, such as red, purple, blue, and yellow, found in plants (Markham, 1988). Flavonoids comprise several main groups, including anthocyanins, flavanols, and flavones, which are widely distributed in plants, while chalcones, aurones, flavonols, dihydrochalcones, and isoflavones are limited to specific groups (Harborne, 1987). Flavonoids are polar because they form glycosides and bind to sugars. Polar solvents such as ethanol, methanol, water, and semi-polar solvents such as ethyl acetate can extract flavonoids from plants (Ekawati et al., 2017). The extraction of active compounds from plants can be influenced by several factors, including the choice of solvent and extraction method (Suryani et al., 2015). The choice of solvent type is adjusted to the polarity of the desired compound, based on the principle of like dissolves like, meaning a solvent will dissolve compounds that have the same level of polarity (Anggitha, 2012). The solvents used in this study were 70% ethanol and ethyl acetate.

The extraction method used in this study was maceration. Maceration involves soaking plant samples, which results in the breakdown of cell membranes due to pressure differences between the

inside and outside of the cells (Koirewoa et al., 2012). Based on this background, the researchers were interested in examining the total flavonoid content of lamtoro leaves using polar solvents, namely 70% ethanol, and semi-polar solvents, namely ethyl acetate, of lamtoro (*Leucaena leucocephala* (Lam.) de Wit) leaf extract using UV-Vis spectrophotometry. Many studies have focused solely on the flavonoid content of lamtoro (*Leucaena leucocephala* (Lam.) de Wit) leaf extract. To the author's knowledge, no researchers have compared the total flavonoid content in 70% ethanol and ethyl acetate extracts of lamtoro (*Leucaena leucocephala* (Lam.) de Wit) leaves.

METHODS

Type of Research

The type of research conducted is experimental, namely the Difference in Determination of Total Flavonoid Content of 70% Ethanol Extract and Ethyl Acetate of Lamtoro Leaves (*Leucaena* leucocephala (Lam.) de Wit) Using the UV-Vis Spectrophotometry Method.

Preparation of Leucaena Leaf Simplex

Three kilograms of leucaena leaves were wet sorted, washed with clean running water, then finely chopped to dry quickly. They were dried in a drying cabinet at 500°C. The dried leucaena leaves were reweighed to calculate the percentage of drying loss. They were then powdered by blending and sifting through a No. 40 sieve (Kusriani, R.H., & Zahra, 2015). The moisture content of the leucaena (*Leucaena leucocephala* (Lam.) de Wit) leaf simplex was determined using a moisture balance. One gram of finely powdered leucaena (*Leucaena leucocephala* (Lam.) de Wit) leaves was placed into the moisture balance. This process was replicated three times, and the average results were calculated (Ramadhani, 2020).

Preparation of 70% ethanol and ethyl acetate extract of Leucaena leaves

Weigh 100 grams of dry powder and place it in a maceration bottle, add 1000 ml of 70% ethanol (1:10). Fill the second bottle with 100 grams of dry powder and add 1000 ml of ethyl acetate (1:10) until the entire sample is submerged. The maceration is carried out for 24 hours, stirring occasionally. Re-maceration is carried out three times until the solution becomes clear (Asmorowati & Lindawati, 2019). The resulting liquid extract is thickened with a rotary evaporator at 400°C (Amelinda et al., 2018), and the yield is calculated.

Data Collection Techniques

a) Preparation of a standard quercetin solution
   Weigh 25 mg of quercetin into a 25 ml volumetric flask and dissolve it in ethanol p.a. to the mark. This results in a concentration of 1000 ppm (Sari et al., 2019).
b) Determination of the Maximum Wavelength (ƛ Max)
   1 ml of a 60 ppm quercetin solution is taken, added with 0.1 ml of 10% AlCl3 and 0.1 ml of 1 M sodium acetate, then ethanol p.a. to the mark in a 10 ml volumetric flask. The maximum wavelength of quercetin is then calculated by running the standard quercetin solution over a wavelength range of 400-800 nm until the maximum wavelength of absorbance is obtained (Sari et al., 2019).
c) Determining Operating Time
   Operating time (OT) was determined by taking 1.0 ml of a 60 ppm quercetin solution and placing it in a test tube. 0.1 ml of 10% aluminum (III) chloride (AlCl3) and 0.1 ml of 1M sodium acetate were added, followed by ethanol p.a. to the mark (10 ml). The absorbance of the solution was measured at the maximum wavelength obtained at 1-minute intervals for 1 hour. A stable absorbance value indicates the operating time (Sari et al., 2019).
d) Standard Curve Preparation
   1.0 ml of each standard quercetin solution at concentrations of 20, 40, 60, 80, and 100 ppm was taken into a 10 ml volumetric flask. 0.1 ml of 10% AlCl3, 0.1 ml of sodium acetate, and ethanol p.a. were added to the mark (10 ml). The mixture was allowed to stand for the operating time and measured at the maximum wavelength obtained. The absorbance measurement results were graphed between the quercetin concentration (on the X-axis) and the absorbance value (on the Y-axis), resulting in a linear equation of $y = bx + a$ (Sari et al., 2019).
e) Determination of Total Flavonoid Content of Ethanol Extract of *Leucaena leucocephala* (Lam.) de Wit Leaves
   50 mg of the thick extract was weighed and dissolved in ethanol p.a. up to 50 ml (solution concentration 1000 ppm). The ethanol extract test solution in a 1.0 ml pipette was added with 0.1

ml of 10% aluminum (III) chloride ($AlCl_3$), added with 0.1 ml of 1 M sodium acetate, then with ethanol p.a. to the limit mark (10 ml), shaken until homogeneous. After that, the solution was incubated at room temperature for the operating time and measured on a spectrophotometer with the maximum wavelength obtained (Sari et al., 2019).

Data Analysis

The data obtained from this study were primary data analyzed using the absorbance data from the standard curve, and then a calibration curve was created. The next step was to calculate the total flavonoid content using the linear regression equation y = bx + a, obtained from the comparative calibration curve (Pertiwi et al., 2021).

RESULTS AND DISCUSSION

Determination of Maximum Wavelength

The UV-Vis spectrophotometry measurements yielded a wavelength of 424 nm with an absorbance of 0.583 nm. According to a journal (Sari et al., 2019), the maximum wavelength is 412 nm. Differences in wavelength results may be due to differences in the instruments used, as each instrument has varying sensitivity and accuracy (Suhartati Tati, 2017). The wavelength results can be seen in Table 1.

Table 1. Maximum Wavelength

Wavelength	absorbance
421	0,571
422	0,575
423	0,579
424	0,583
425	0,566
426	0,568
427	0,569
428	0,569

Source: Processed primary data (2023)

Determining Operating Time

The measurement results showed a stable time at 22 minutes with an absorbance of 0.571. The operating time results can be seen in Table 2.

Table 2. Operating Time

Time	absorbance
1200	0,572
1260	0,570
1320	0,571
1380	0,571
1440	0,571
1500	0,571
1560	0,571
1620	0,571
1680	0,571

Source: Processed primary data(2023)

Results of standard curve absorbance measurement of quercetin

The results of the standard curve measurements are presented in Table 3.

Table 3. Standard Curve Absorbance Measurement Of Quercetin

Time	absorbance	Linear Regression Equation
20	0,306	a = 0,1949
40	0,414	b = 0,0053
60	0,506	R2 = 0,9943
80	0,605	
100	0,744	y = bx + a
		y = 0,0053x + 0,1949

Source: Processed primary data(2023)

Results of the study on total flavonoid levels

The total flavonoid levels of the 70% ethanol and ethyl acetate extracts were calculated using linear regression from the previously measured quercetin standard curve. The results of the quercetin and flavonoid equivalence values are shown in Tables 4 and 5.

Table 4. Results of Determination of Total Flavonoid Content of Ethanol Extract

Concentration (ppm)	Replication	Absorbance	Rate Ekuivalen (ppm)	Rate Flavonoid Total (%)
1000	1.	0,492	56,06	5,61%
	2.	0,475	52,84	5,28%
	3.	0,484	54,55	5,46%
Average			54,48	5,45% ±0,17

Source: Processed primary data(2023)

Table 5. Results of Determination of Total Flavonoid Content of Etil asetat Extract

Concentration (ppm)	Replication	Absorbance	Rate Ekuivalen (ppm)	Rate Flavonoid Total (%)
1000	1.	0,575	71,72	7,17%
	2.	0,545	66,06	6,61%
	3.	0,568	70,40	7,04%
Average			69,39	6,94% ±0,29

Source: Processed primary data(2023)

Result Independent T-Test Kadar Flavonoid Total

Table 2. Result Independent T-Test Kadar Flavonoid Total

Test Statistics	
	Flavonoid
Sig (2-tailed)	0,002
Df	4
t	-7,671

Source: Processed primary data(2023)

Based on the results of the Independent T-Test of total flavonoid content data using SPSS, the significance value obtained can be seen in Table 9.3. In the table, based on test statistics, the Sig. Value is 0.002, meaning that if the sign <0.05, there is a significant difference in this study.

CONCLUSION

The secondary metabolites contained in the 70% ethanol and ethyl acetate extracts using the phytochemical screening method, were flavonoids, alkaloids, tannins, and saponins. The total flavonoid content of the 70% ethanol extract of lamtoro leaves was 5.44% and the total flavonoid content of the ethyl acetate extract of lamtoro leaves was 6.94%. There was a significant difference between the flavonoid content of the 70% ethanol extract and the ethyl acetate extract of lamtoro leaves because the significance value was <0.05.

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