Pharmaceutical Reports

Research Article

Determination of Phenolic and Flavonoid Content of Ethanol Extract of Manila Sawo Leaf (*Manilkara zapota* L.) from Wajo District by Uv-Vis Spectrophotometry

Asriani Suhaenah, Suki Hartina, Aminah*

Laboratory of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Muslim Indonesia, Makassar 90231, Indonesia
* Corresponding Author. E-mail: aminah.aminah@umi.ac.id.

Received: 09 April 2024 / Revised: 19 May 2024 / Accepted: 21 July 2024

ABSTRACT: Sapodilla Manila (*Manilkara zapota* L.) is a plant that is widely known by the public. People usually use sapodilla leaves as traditional medicine. Efficacy obtained from the presence of phenolic compounds and flavonoids in these plants. This study aims to determine the phenolic and flavonoid content of ethanol extract in sapodilla leaves (*Manilkara zapota* L.) from Wajo district. This research method used UV-Visible spectrophotometry and the reference standard used were gallic acid (phenolic) and quarcetin (flavonoid). The results showed that the average phenolic content of the ethanol extract of sapodilla leaves at a concentration of 100 ppm was 150.28 mgGAE/g extract and the average flavonoid content of the ethanol extract of sapodilla leaves at a concentration of 300 ppm was 36.577 mgQE/g extract.

KEYWORDS: Sapodilla leaf manila (*Manilkara zapota* L.); phenolics; flavonoids; UV-Vis Spectrophotometer.

1. INTRODUCTION

Indonesia is famous forrichesnature which has various types of plants that are efficacious as medicine. Traditional medicine has been known and used for generations by Indonesian people. Communities far from health services generally use plants as medicine (Bahriul et al., 2014).

One of these plants is the Sapodilla manila plant which is widely known by the public, usually used as a shade, the sap for the manufacture of chewing gum, the leaves are used as a medicine for diarrhea, fever, cough, antimicrobials, antioxidants and antibiotics. The wood is useful for building materials. The flowers can also be used as ingredients for cosmetics and most commonly the fruit can be consumed and processed foods (Hasanah et al., 2019). Especially sapodilla leaves contain active substances such as saponins, tannins and flavonoids. Saponins are able to inhibit bacterial growth by inhibiting protein synthesis and reducing the surface tension of bacterial cells resulting in leakage. Tannins work by lysing the bacterial cell walls. While flavonoids inhibit DNA synthesis and energy metabolism of bacteria (Mufti et al., 2017).

According to research conducted by Ratna Widyasari (2021), active metabolite compounds such as phenolics, saponins, tannins and flavonoids, have antibacterial activity. Flavonoids are proven to have very strong biological effects as antioxidants, inhibit the clumping of blood cell fragments, stimulate the production of nitrites which can dilate blood vessels, and also inhibit the growth of cancer cells (Fawwaz et al., 2023). Flavonoids include natural phenolic compounds that have potential as antioxidants and have bioactivity as drugs. These compounds which can be found in stems, leaves, flowers and fruit are red, purple and blue dyes as well as some yellow dyes.

The ability of phenolic compounds as biologically active compounds plays a major role in human interests. One of them is as an antioxidant for the prevention or treatment of degenerative diseases such as cancer, premature aging and disorders of the body's immune system. Determination of phenolic content can be carried out using the UV-Vis Spectrophotometry method because the hydroxyl groups in the phenolic component with the Folin Ciocalteu reagent produce a blue color which can be detected by UV-Vis spectrophotometry (Supriningrum et al., 2020).

Based on the above, this research was carried out, entitled Determination of phenolic and flavonoid levels from the ethanol extract of sapodilla leaves (*Manilkara zapota* L.) using the UV-Vis spectrophotometry method. (Sari & Ayuchecaria, 2017).

2. EXPERIMENTAL SECTION

2.1. Population and Sample

The sample population was the sapodilla plant (*Manilkara zapota* L.) sourced from Wajo District, South Sulawesi Province and the sample used was the ethanol extract of sapodilla leaves (*Manilkara zapota* L.).

2.2. Materials and tools

The materials used in this study arealuminum foil, aluminum chloride ($AlCl_3$) 96% ethanol, gallic acid pa (Sigma), distilled water (Brataco Chemica), ethyl acetate, Na_2CO_3 7%, Folin ciocalteau pa reagent (Merck), ethanol extract of manila sapodilla leaves (*manilkara zapota* L.), quettetin, labels, 1 M potassium acetate (1 M CH_3CO_2K), ferric chloride ($FeCl_3$), magnesium sulfate (Mg), and hydrochloric acid (HCI). While the tools used are a set of extraction tools

with the maceration method, blender (Philips), oven, analytical scales (Ohaus), jars, stirring rods, filter paper, glass funnels, Erlenmeyer, measuring cups, beakers, evaporating cups, rotary evaporator (Ika® RV 10 basic), water bath (memmert) and separatory funnel. Tools for in vitro testing include measuring flasks, test tubes, 1000 µL micropipette, measuring pipette, dropping pipette, spatula, vials, cuvettes, and Ultraviolet Visible spectrophotometer.

2.3. Qualitative analysis

2.3.1. Test for phenolic compounds

Qualitative test of phenolic compounds was carried out by reacting 1 gram of ethanol extract of sapodilla leaves (*Manilkara zapota* L.) by adding 1% $FeCl_3$ marked with the formation of green, red, purple, blue or dark black, which occurs when $FeCl_3$ reacts with the hydroxyl groups present in phenolic compounds (Princess et al., 2019).

2.3.2. Flavonoid compound test

A total of 3 mg of sample extract was put into a test tube. Then added magnesium powder and 5 drops of concentrated hydrochloric acid. Positive for flavonoids if it produces yellow, orange and red colors (Eaton, 2020).

2.4. Phenolic Quantitative Analysis

2.4.1. Preparation of a standard solution of gallic acid

A standard gallic acid solution of 1000 ppm is prepared by weighing 5 mg of gallic acid dissolved in 96% ethanol to 5 mL. Pipette 50, 75, 100, 125, and 150 µl of the solution and add up to 5 ml of distilled water to produce concentrations of 10, 15, 20, 25 and 30 ppm.

2.4.2. Maximum wavelength determination

Maximum wavelength determination performed using 75 µL of gallic acid solution with a concentration of 15 ppm. Pipette as much as 1 ml of the solution, add 1 ml of Folin Ciocalteu (1:9) then the solution is homogenized and left for 3 minutes. After that, 1 mL of 7% Na_2CO_3 solution was added, then the solution was shaken until homogeneous and the solution was left for 1 hour at room temperature in a closed room. The absorbance of the solution was measured at a wavelength of 400 – 800 nm.

2.4.3. Standard curve creation

Measurement of gallic acid standard solution was carried outby making a series of concentrations of 5, 10, 15, 20, 25 and 30 ppm. As much as 1 ml of each solution was taken and 1 mL of Folin Ciocalteu (1:9) was added, the solution was shaken and allowed to stand for 3 minutes. 1 mL of 7% Na_2CO_3 solution was added, then the solution was shaken until homogeneous. Then the solution was allowed to stand for 1 hour at room temperature in a closed room. The absorbance of the solution was measured at a maximum wavelength of 756 nm and a gallic acid calibration curve was made.

2.4.4. Sample preparation and measurement

Determination of the phenolic content of the ethanol extract of sapodilla leaves was carried out byweigh 10 mg of each extract and dissolve it with 0.5 ml of 96% ethanol and dilute with distilled water to 10 mL and then homogenize to obtain 1000 ppm. From 1000 ppm diluted to 100 ppm, then from 100 ppm pipetted as much as 1 ml of the sample solution was taken and added 1 mL of Folin Ciocalteu, the solution was shaken and left for 3 minutes. 1 mL of Na_2CO_3 was added to the solution, the solution was shaken until homogeneous and left to stand for 1 hour in a closed room. The absorbance of the solution was measured at the maximum wavelength in 3 repetitions.

2.5. Flavonoid Quantitative Analysis

2.5.1. Preparation of Quarcetine Standard Curves

Weighed as much as 5 mg of quercetin standard and dissolved in 5 mL of 96% ethanol (1000 ppm). The stock solution was pipetted as much as 0.5 mL and the volume was made up to 5 mL with ethanol to obtain a concentration of 100 ppm. From a 100 ppm quercetin standard solution, several concentrations were made, namely 12 ppm, 14 ppm, 16 ppm, 18 ppm, and 22 ppm. From each concentration of quercetin standard solution, pipette 1 mL. Then 1 mL of 10% $AlCl_3$ and 1 mL of potassium acetate were added, after which the samples were incubated for 30 minutes at room temperature. The absorbance was determined using the UV-Vis spectrophotometry method at a maximum wavelength of 432 nm (Fawwaz et al., 2022).

2.5.2. Maximum wavelength determination

Determination of the maximum wavelength of quercetin was carried out by running a quercetin solution in the wavelength range of 400 – 800 nm. The results of running show the maximum wavelength of the quercetin standard used to measure the absorption of the sample (Amina et al., 2017).

2.5.3. Sample preparation and measurement

Weighed 10 mg of ethanol extract of sapodilla leaves, dissolved in 10 mL of ethanol, to obtain a concentration of 1000 ppm. From this solution, a dilution of 300 ppm was made. From a 300 ppm solution, 1.5 mL was pipetted and then 1 mL of 10% $AlCl_3$ solution and 1 mL of 1 M potassium acetate were added. Then the samples were incubated for 30 minutes at room temperature. The absorbance was determined using the UV-Vis spectrophotometry method at a

maximum wavelength of 432 nm. Samples were made in three replications for each analysis and the average absorbance value was obtained.

2.6. Data analysis

The resulting data was first processed using the standard curve method; a linear regression y = a + bx was made based on the absorbance and concentration data of the standard solution.

Where : y = Absorbance
x = Concentration © mg/L
b = Slop (slope)
a = intercept

3. RESULTS AND DISCUSSION

Qualitative tests were carried out to determine the chemical components in plants, by using color reactions using certain reagents (Vifta & Advistasari, 2018)**.** The formation of dark green, red, purple, blue or black colors, which occurs when $FeCl_3$ reacts with the hydroxyl groups present in phenolic compounds (Princess et al., 2019). The results of the qualitative test showed that the ethanol extract of sapodilla leaves positively contained phenolic compounds which were indicated by the appearance of a red color. While the identification test for Flavonoid compounds by adding Mg and HCl to flavonoid compounds aims to reduce the benzopiron nucleus contained in the flavonoid structure so that the color changes occur. Positive for flavonoids if it produces yellow, orange and red colors (Eaton, 2020). And the results of the qualitative test showed that the ethanol extract of sapodilla leaves positively contained Flavonoid compounds which were indicated by the formation of an orange color after being reacted with Mg and concentrated HCI. The addition of HCl resulted in an oxidation-reduction reaction between Mg metal as a reducing agent and flavonoid compounds. The results of the qualitative test using color reagents can be seen in **Table 1.**

Table 1. Qualitative test resultsphenolic and flavonoid compounds of the ethanol extract of the leaves of the sapodilla plant (*Manilkara zapota* L.)

Sample	Identification	
	Phenolic ($FeCl_3$)	**Flavonoids (Mg + HCI P)**
Leaves of the manila sapodilla plant (*Manilkara zapota* L.)	(+) Red Color	(+) Orange color

Furthermore, quantitative analysis was carried out to determine the levels of Phenolics and Flavonoids using the visible area spectrophotometry method. This method was chosen because it can be used for the analysis of a substance in small quantities, the process is easy, simple, quite sensitive and selective, and also has a fairly high analytical sensitivity. In addition, the presence of chromoform and ausochrome groups in phenolic compounds and flavonoids is the fundamental reason for using the spectrophotometric method (Estikawati & Lindawati, 2019).

In determining the phenolic content, a standard solution, namely gallic acid, is used because gallic acid is one of the natural phenols that has strong antioxidant activity and is a stable substance and is a compound that is commonly used as a standard in determining the content of phenolic compounds (Pallawagau et al., 2019).

The Folin-Ciocalteau reagent is used as a reagent because phenolic compounds can react with Folin-Ciocalteau to form a colored solution whose absorbance can be measured. The principle of this method is the formation of a blue complex compound which can be measured at the maximum wavelength. Determination of phenolic content was carried out using the Folin-Ciocalteau reagent which contained a mixture of sodium tungstate, sodium molybdate, lithium sulfate, concentrated hydrochloric acid, 85% phosphoric acid, bromine, and distilled water. When the sample was added with the Foiln-Ciocalteau reagent, this reagent oxidized phenolic (alkaline salts) or phenolic-hydroxy groups of heteropoly acids (phosphomolybdate-phosphogstat) present in the Folin-Ciocalteau reagent to a molybdenum-tungsten complex. Phenolic compounds react with Folin Ciocalteau reagent only in alkaline conditions so that proton dissociation occurs in phenolic compounds to become phenolic ions. To obtain an alkaline atmosphere, 7% Na_2CO_3 is used. The greater the concentration of phenolic compounds, the more phenolic ions will reduce heteropoly acids (phosphomolybdate-phosphotungstate) into molybdenum-tungsten complexes so that the resulting blue color becomes more intense (Andriani & Murtisiwi, 2018).

Prior to measurement, a standard solution of gallic acid was first measured at the maximum wavelength at a concentration of 15 ppm with a wavelength range between 400-800 nm. The result of determining the maximum wavelength is at a wavelength of 756 nm, where at this wavelength the absorbance of gallic acid shows the maximum absorbance. Determination of the maximum wavelength aims to determine the size of the wavelength needed for gallic acid solution to achieve maximum absorption (Fatonah et al., 2021).

In measuring phenolic content, measurements of standard gallic acid or concentration series are first carried out, in which aquadest is added first. Aquadest is used because what will be analyzed is phenolic compounds which are compounds that can dissolve in distilled water. Then react withFolin-Ciocalteau and Na_2CO_3, the two reagents were added as complexing agents. The purpose of adding complexing agents is to make the solution colored so that it can be analyzed using UV-Vis spectrophotometry with the aim of shifting the wavelength towards visible. The solution whose absorption had been observed by UV-Vis spectrophotometry with λ maximum 756 nm.

The concentration series used in this test were 10, 15, 20, 25 and 30 ppm. After being measured using UV-Vis spectrophotometry, the results can be seen in **Table 2**. After that, the absorbance data from each concentration series that has been obtained, is then entered into the Microscoft excel application in order to obtain the appropriate standard curve along with its r value.

Table 2. Resultsmeasuring the absorbance of a standard solution of gallic acid

Concentration (ppm)	absorbance
10	0.290
15	0.486
20	0.681
25	0.792
30	0.969

After measuring the absorbance, a graph of the standard gallic acid curve was made to obtain a linear regression equation. The graph of the gallic acid standard curve can be seen in **Figure 1**.

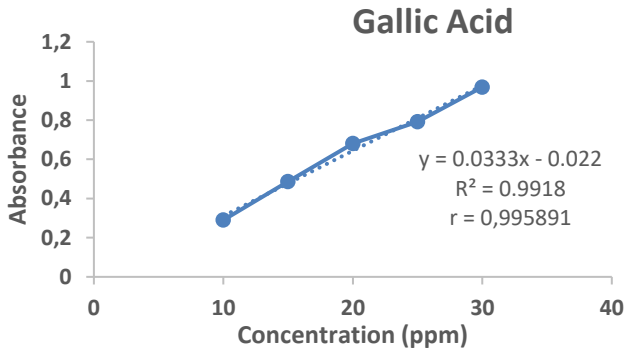

Figure 1. Linear regression of gallic acid

The results obtained in the series of measurements of the concentration of standard gallic acid solutions in **Table 3**, are plotted against the concentration so that the value of the coefficient of determination (R2) is 0.9918, the value of the standard calibration curve at the correlation coefficient (r) is 0.995, the value of the standard curve of the calibration curve in this test meet the requirements according to the stipulated conditions, namely r≥0.995 (Suryani et al., 2022). As in the picture above, a linear regression equation y = 0.0333x – 0.022 is obtained, which can be used to determine the phenolic content of the ethanol extract of sapodilla leaves (*Manilkara Zapota* L.).

After standard measurement of gallic acid was carried out, measurements were carried out on the test sample with a concentration of 100 ppm, which was weighed as much as 10 mg made 3 times of replication, where the measurement results of the test samples were then plotted with the results of measurements of gallic acid so that the phenolic content of the ethanol extract of sapodilla leaves was obtained (*Manilkara Zapota* L.). The results of the calculation of the determination of phenolic content in the ethanol extract of sapodilla leaves (*Manilkara Zapota* L.) can be seen in **Table 3.**

Table 3. The results of determining the phenolic content of the ethanol extract of sapodilla leaves (*Manilkara zapota* L.)

Replication	Abs (Y)	Extract weight (mg/L)	Total phenolic content (mgGAE/g extract)	Average phenolic content (mgGAE/g extract)
I	0.788	0.0166	146.530	
II	0.728	0.0166	135.674	150.28
III	0.798	0.0146	168.657	

Based on the results of the study, in table 3 the phenolic content of the ethanol extract of sapodilla leaves (*Manilkara zapota* L.) is150.28 mgGAE/g extract means that every 1 gram of ethanol extract of sapodilla leaves is equivalent to 150.28 mg of gallic acid.

Meanwhile, to determine the levels of Flavonoid compounds in the sample, quercetin was used as a standard solution, because quercetin is a flavonol class of flavonoids which has a keto group on the C-4 atom and also a hydroxyl group on neighboring C-3 and C-5 atoms. In this study, to determine the levels of flavonoids in the sample, quarcetin was used as a standard solution with a concentration series of 12, 14, 16, 18 and 22 ppm. The concentration series is used because the method used to determine the concentration is a method that uses the standard curve equation. To create a

standard curve, several concentration series are first made to obtain a linear equation that can be used to calculate the percent content (Amina et al., 2017).

Prior to measurement, a standard quercetin solution was first measured at the maximum wavelength at a concentration of 18 ppm with a wavelength range between 400-800 nm. The result of determining the maximum wavelength is at a wavelength of 432 nm, where at this wavelength the absorbance of quarcetin shows maximum absorbance.

In measuring the levels of flavonoids, measurements of standard quercetin or concentration series were first carried out, in which aquadest was added first. Aquadest is used because what will be analyzed is phenolic compounds which are compounds that can dissolve in distilled water. Then react with $AlCl_3$ and Potassium acetate, the addition of these reagents, in which the function of the $AlCl_3$ reagent is to form a reaction between $AlCl_3$ and the flavonoid group to form complexes between neighboring hydroxyl and ketone groups or with neighboring hydroxyl groups. $AlCl_3$ will react with the ketone group on C4 and the OH group on C3 or C5 in flavones or flavonols to form a stable yellow complex compound. The addition of potassium acetate is to detect the presence of 7-hydroxyl groups (Jubaidah, 2018). The solution whose absorption had been observed by UV-Vis spectrophotometry with λ maximum 432 nm.

The concentration series used in the quarcetin standard test were 12, 14, 16, 18 and 22 ppm. After being measured using UV-Vis spectrophotometry, the results can be seen in **Table 4**. After that, the absorbance data from each concentration series that has been obtained, is then entered into the Microsoft Excel application in order to obtain the appropriate standard curve along with its r value.

Table 4. Results of absorbance measurements of quercetin standard solutions

Concentration (ppm)	absorbance
12	0.204
14	0.229
16	0.278
18	0.320
22	0.420

After measuring the absorbance, a graph of the quarcetin standard curve was then made to obtain a linear regression equation. Quarcetin standard curve graph can be seen in **Figure 2.**

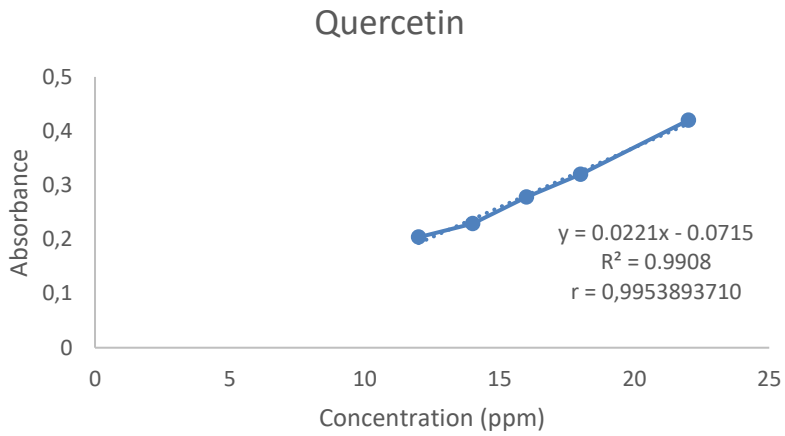

Figure 2. Linear regression of quercetin

At a wavelength of 432 nm from the calibration curve, a linear regression equation is obtained, namely y = 0.0221x – 0.0715 with a correlation coefficient (r) of 0.9908. This equation was used to determine the levels of flavonoids in the ethanol extract of sapodilla leaves *(Manilkara zapota* L.). After measuring the standard quercetin, measurements were then taken on the test sample with a concentration of 300 ppm which was weighed as much as 10 mg, made 3 times of replication, where the measurement results of the test samples were then plotted with the results of the quercetin measurements so that the levels of flavonoids from the ethanol extract of sapodilla leaves (*Manilkara Zapota* L.) were obtained. The results of calculating the determination of flavonoid levels in the ethanol extract of sapodilla leaves (*Manilkara zapota* L.) can be seen in **Table 5**.

Table 5. The results of determining the levels of flavonoids in the ethanol extract of sapodilla leaves (*Manilkara zapota* L.)

Replication	Abs (Y)	Extract weight (mg/L)	Total content of flavonoids (mgQE/g extract)	Average content of flavonoids (mgQE/g extract)

I	0.248	0.0141	34.143	
II	0.277	0.0140	37.507	36.577
III	0.295	0.0145	38.083	

In this study, the flavonoid content of the ethanol extract of sapodilla leaves (*Manilkara zapota* L.) from Wajo district by UV-Vis spectrophotometry was 36.577 mgQE/g extract, meaning that in every 1 gram of ethanol extract of sapodilla leaves (*Manilkara zapota* L.) is equivalent to 36.577 mg quercetin.

4. CONCLUSION

Based on the results of the research conducted, it can be concluded that the phenolic content of the ethanol extract of sapodilla leaves (*Manilkara zapota* L.) was 150.28 mgGAE/g extract and the flavonoid content of the ethanol extract of sapodilla leaves (*Manilkara zapota* L.) was 36.577 mgQE/g extract

Acknowledgments: The authors are grateful to The Head of Pharmaceutical Chemistry Laboratory, Universitas Muslim Indonesia. The authors are thankful to The Dean of Faculty of Pharmacy Universitas Muslim Indonesia for the space to do this research.

Author contributions: All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

Conflict of interest: The authors declared no conflict of interest.

Ethical Approval: Not applicable

REFERENCES

Aminah, A., Tomayahu, N., & Abidin, Z. Determination of total flavonoid content of ethanol extract of avocado peel (Persea americana mill.) Using uv-vis spectrophotometry method. Indonesian Phytopharmaca Journal, 2017: 4(2), 226–230.

Andriani, D., & Murtisiwi, L. Determination of Total Phenolic Content of Ethanol Extract of Butterfly Pea Flower (*Clitoria Ternatea* L.) Using Uv Vis Spectrophotometry. Scholar Journal of Pharmacy, 2018: 2(1), 32–38.

Bahriul, P., Rahman, N., & Diah, AWM. Antioxidant Activity Test of Bay Leaf Extract (*Syzygium polyanhum*) Using DPPH. Journal of Academic Chemistry, 2014: 3(3), 143–149.

Eaton, DC. The World of Organic Chemistry. MC. Graw-Hill Book Company. 2020: 6(1).

Estikawati, I., & Lindawati, NY. Determination of Total Flavonoid Content of Oyong Fruit (*Luffa acutangula* (L.) Roxb. Using UV-Vis Spectrophotometry Method. Journal of Science And Practical Pharmacy, 2019: 5(2), 96–105.

Fatonah, R., Mulyaningsih, S., & Ardiana, C. Determination of Total Tannin Content from Binahong Leaf Extract (Anredera cordifolia). Journal of Life Science: Journal of Education and Natural Sciences, 2021: 3(2), 38–46.

Fawwaz M, Pratama M, Musafirah, Wahab I, Iriani R, Aminah, Kusuma AT, Arsul MI. Evaluation of Antioxidant Activity of *Vernonia amygdalina* Leaves and Its Flavonoid-Phenolic Content. Indonesian Journal of Pharmaceutical Science and Technology. 2023; 10(2): 104-110.

Fawwaz M, Muflihunna A, Pratama M, Rahmawati R, Razak R, Baits M. Total phenolic and flavonoid compound of crude and purified extract of green tea leaves (*Camellia sinensis*) from Makassar-Indonesia. Jurnal Fitofarmaka Indonesia. 2022; 9(3): 19-24.

Hasanah, N., Kardhinata, EH, & Nasution, J. Anti Bacterial Test of Sapodilla Leaf Extract (*Manilkara zapota*) Against Escherichia coli. UMA Scientific Journal of Biology (JIBIOMA), 2019: 1(2), 58–63.

Jubaidah, SJ. Analysis Of Total Flavonoids Of The Root Of Tabar Kedayan (*Aristolochia foveolata* Merr). Al-Kimia, 2018: 6(2).

Pallawagau, M., Yanti, NA, Jahiding, M., Kadidae, LO, Asis, WA, & Hamid, FH. Determination of Total Phenolic Content of Liquid Volatile Matter from Pyrolysis of Cocoa Fruit Peel and Antifungal Activity Test against Fusarium oxysporum. ALCHEMY Journal of Chemical Research, 2019: 15(1), 165.

Putri, HD, Sumpono, S., & Nurhamidah, N. Liquid Smoke Activity Test of Rubber (Hevea Brassiliensis) Fruit Cells And Its Application In Inhibiting Beef's Tendition. Allotropes, 2019: 2(2), 97–105.

Sari, AK, & Ayuchecaria, N. Determination of Total Phenolic and Total Flavonoid Content of Black Rice Extract (*Oryza Sativa* L.) from South Kalimantan. Scientific Journal of Ibn Sina, 2017: 2(2), 327–335.

Supriningrum, R., Nurhasnawati, H., & Faisah, S. Determination Of Total Phenolic Content Of Serunai Leaf Ethanol Extract Using Uv-Vis Spectrophotometry Method. Al Ulum Journal of Science and Technology, 2020: 5(2), 54.

Suryani, MY, Paramita, A., Susilo, H., & Maharsih, IK. Analysis of Determination of Iron (Fe) Content in Coal Mine Wastewater Using a UV-Vis Spectrophotometer. Indonesian Journal of Laboratory, 2022: 5(1), 7.

Vifta, RL, & Advistasari, YD. Phytochemical Screening, Characterization, and Determination of Total Flavonoid Content of Parijoto Fruit Extract and Fractions (*Medinilla speciosa* B.). Proceedings of Unimus National Seminar, 2018: 1, 8–14.

Widyasari, R., & Yuspita Sari, D. Determination of Total Flavonoid Content of Ethanol Extract of Sapodilla (*Manilkara zapota* (L.) Stem Bark by UV-Visible Spectrophotometry. Journal of Indonesian Pharmacists, 2021: 4(2), 237–244.

Wijaya, H., Novitasari, & Jubaidah, S. Comparison of Extraction Methods to the Yield of Sea Rambui Leaf Extract (*Sonneratia caseolaris* L. Engl). Manuntung Scientific Journal, 2018: 4(1), 79–83.