

DETERMINATION OF TANNIN CONTENT IN ETHANOL EXTRACT OF WATER GUAVA (*Syzygium aqueum*) LEAVES USING THE PERMANGANOMETRIC METHOD

Nabillah Vichria Aliyasir^{1*}, Wa Ode Syafriah², Ratih Nurwanti³

^{1,2,3} Politeknik Baubau, Baubau, Indonesia

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CORRESPONDING AUTHOR

Name: Wa Ode Syafriah

Address: Baubau

E-mail : waodesyafriah@gmail.com

ABSTRACT

Background Water guava (*Syzygium aqueum*) leaves are one of the traditional medicinal plants that contain various secondary metabolites, one of which is tannins. Tannins have activities as astringent, antidiarrheal, antibacterial, and antioxidant agents, making it important to determine their content. These compounds are widely utilized in the pharmaceutical field as well as in traditional medicine because of their significant physiological effects. The purpose of this study was to determine the tannin content in the ethanol extract of water guava (*Syzygium aqueum*) leaves. **Objective:** This study aimed to identify the presence of tannins qualitatively and to determine the tannin content in the ethanol extract of water guava leaves using the permanganometry method. The permanganometry method used in this research is a redox titration method employing potassium permanganate (KMnO_4) solution as an oxidizing agent. Water guava leaves were extracted using the maceration method with 96% ethanol solvent to draw out the active compounds. Qualitative testing was conducted using FeCl_3 reagent, indicated by a color change, while quantitative testing was carried out through permanganometric titration using KMnO_4 solution until the endpoint was marked by a distinct color change. **Results:** The results of this study showed that the ethanol extract of water guava leaves tested positive for tannins, indicated by a greenish-black color in the qualitative test, while the quantitative test showed a pale pink color with a tannin content of 27.91%. **Conclusion:** These findings demonstrate that water guava leaves contain a relatively high level of tannins and have the potential to be utilized as a natural source of compounds beneficial in the health sector as well as in further research related to phytopharmaceutical development.

INTRODUCTION

Indonesia, which is located in the tropical region, is known for its abundant natural resources in the form of plants that can be utilized as raw materials for medicine to treat various types of diseases, making Indonesia one of the largest users of medicinal plants in the world. The use of plants as traditional medicine in Indonesia has been practiced for thousands of years and has become an important part of the community's natural healing heritage (Yassir & Asnah, 2019). Water guava leaves contain various active compounds such as tannins, flavonoids, essential oils, and alkaloids. The tannins present in these leaves have chelating properties and act as spasmolytic agents, which help contract the intestinal muscles, thereby reducing peristaltic movement. In addition, tannins can also damage bacterial cell membranes, disrupting the balance and function of the cells ((Bawamenewi et al., 2023).

Tannins are organic compounds in the form of complex polyphenol mixtures composed of carbon (C), hydrogen (H), and oxygen (O) elements, generally large molecules with high molecular weight, and are chemically classified into hydrolyzable tannins and condensed tannins (Matoa & Spektrofotometri, 2022). Active compounds in plants can be extracted depending on the type of solvent used, where the solvent plays a significant role in the amount of compounds such as flavonoids, saponins, and tannins obtained, while also influencing the characteristics of the final extract such as specific gravity, moisture content, and ash content (Sari, 2024). Water guava (*Syzygium aqueum*) is a plant from the Myrtaceae family with a height of about 5–10 meters, a short-branched trunk, irregular leaf canopy, elliptical pointed leaves, flowers located at the tips of branches or leaf axils, and fruits with a distinctive aroma containing 1–6 seeds with varying colors (Anggraheni et al., 2019). This plant has wide-ranging benefits, as its bark, seeds, and leaves possess anti-diarrheal, anti-asthmatic, antipyretic, digestive, and therapeutic activities for diabetes, cholesterol, and breast cancer; particularly, the leaves function as an astringent that is beneficial for skin care, such as tightening, reducing pores, and protecting the skin (Restu et al., 2023). Furthermore, selecting the appropriate

type of solvent is an important factor in obtaining the optimal levels of active compounds from plant extraction (Yuliani & Rasyid, 2019).

Extraction is a separation method based on differences in solubility within two immiscible liquids, usually using water and an organic solvent as the medium. In practice, there are various extraction techniques that can be applied, one of which is the maceration method, which is quite popular because it is carried out by soaking plant powder in a suitable solvent inside an inert, tightly closed container at room temperature, making the process simple yet effective (Badaring et al., 2020). In addition, in chemical analysis, there is also the permanganometric method, which is a type of redox titration involving reduction and oxidation reactions. The permanganometric method is often chosen because it has several advantages, including functioning as a strong oxidizing agent, not requiring an additional indicator, being easily available on the market, and being relatively inexpensive. Nevertheless, this method also has an important limitation, namely that the potassium permanganate solution used is unstable if stored for too long, thus requiring periodic standardization to ensure the accuracy of analytical results (Putra & Sugiarso, 2016).

The duration of extraction plays an important role in the outcome, as a process that is too short may not extract all active compounds optimally, while an excessively long process can cause damage to bioactive compounds due to oxidation. This condition occurs because the cavitation mechanism in the ultrasonic method is able to break down cell walls and generate energy that increases temperature, thereby potentially accelerating the oxidation of extracted compounds (Miradita Lestari et al., 2020). Several factors influence the extraction process, including operating temperature, stirring speed, particle size and shape, solid conditions, as well as the type and amount of solvent used. During extraction, mass transfer occurs as the main physical phenomenon, driven by differences in concentration between phases. The greater the concentration difference, the faster the mass transfer occurs until equilibrium is reached (Anggista et al., 2019).

Based on research conducted by Mulyani (2022), the tannin content in pagoda leaf extract (*Clerodendrum paniculatum*) has been analyzed using two methods, namely visible spectrophotometry and permanganometric titration. The extraction process was carried out using the maceration method with 96% ethanol as the solvent on pagoda leaves that had been previously dried. A qualitative test was performed by adding 1% FeCl_3 solution, which showed a bluish-black color change as an indication of the presence of tannin compounds in the extract. Therefore, I chose the permanganometric method for my research because it is simple, does not require expensive equipment like the UV-VIS spectrophotometric method, and has also been widely used in other studies to measure tannin compounds.

METHOD

Time and Place of Research

The purpose of this study was to determine the tannin content contained in the ethanol extract of water guava (*Syzygium aqueum*) leaves. The permanganometric titration method was used, which is simple, practical, and provides a fairly high level of accuracy. To obtain comprehensive information about the tannin content in the sample, the research methodology combined both quantitative and qualitative techniques. The study was conducted systematically and under controlled conditions at the Pharmacy Laboratory of Bina Husa Polytechnic, Kendari, on June 12, 2025.

Tools and Materials

The tools used in this study were a blender, 30-mesh sieve, digital balance, hot plate, magnetic stirrer, glass rod, measuring cylinder, volumetric pipette, dropper pipette, glass funnel, 250 mL volumetric flask, filter paper, Erlenmeyer flask, 25 mL brown burette, thermometer, glass wool, and a Moisture Content Balance. In addition, the materials used in this study were young green water guava (*Syzygium aqueum*) leaves, 96% ethanol, distilled water, KMnO_4 , oxalic acid, H_2SO_4 , indigocarmine, and FeCl_3 .

Sample Preparation

From the water guava (*Syzygium aqueum*) plant, young green leaves were collected. The obtained leaves were then wet-sorted, washed, and drained immediately. The leaves were air-dried until dry and then subjected to dry sorting. Afterward, the dried water guava leaves were ground and sieved using a 30-mesh sieve.

A total of 500 grams of water guava (*Syzygium aqueum*) leaf powder was placed into a maceration container. Then, 2 liters of 96% ethanol were added until the simplicia powder was completely submerged and left to stand for 3–4 days. The resulting macerate was then concentrated using a rotary evaporator to obtain a thick extract.

Qualitative Test

Weigh 1 gram of FeCl_3 powder, dissolve it in approximately 50 mL of distilled water, transfer into a 100 mL volumetric flask, then add distilled water up to the calibration mark and homogenize. Take 0.5 mL of the ethanol extract of water guava (*Syzygium aqueum*) leaves and add 2–3 drops of 1% FeCl_3 solution. The formation of a greenish-black color indicates the presence of tannins.

Indigocarmine Reagent Solution

A total of 6 grams of indigocarmine was dissolved in 500 mL of distilled water and heated. After cooling, distilled water was added to make up to one liter, then the solution was filtered.

KMnO₄ Solution

Weigh 3.2 grams of KMnO_4 and dissolve it in 1 liter of distilled water. Boil the solution for 10–15 minutes, then store it overnight. Afterward, filter and dilute it with distilled water to 1 liter. The standard KMnO_4 solution must be standardized before use.

Standardization of KMnO₄ Solution

Weigh 0.63 grams of oxalic acid crystals and dissolve them in 100 mL of distilled water. Take 25 mL of the oxalic acid solution, add 5 mL of concentrated H_2SO_4 , and heat to 70°C. While still hot, titrate with the standard potassium permanganate (KMnO_4) solution until a purple color appears and the permanganate drops no longer disappear. Record the titration volume.

Determination of Tannin Content 0.01 N

Weigh 1.5 g of the ethanol extract of water guava (*Syzygium aqueum*) leaves, place it into a 100 mL beaker, and add 50 mL of distilled water. Heat the solution at 40–60°C for 30 minutes. After cooling, filter the solution into a 250 mL volumetric flask, then add distilled water up to the calibration mark. Take 25 mL of the solution, transfer it into an Erlenmeyer flask, and add 20 mL of indigocarmine solution. Titrate with 0.01 N KMnO_4 solution until the endpoint is reached, indicated by the solution turning pale pink. The test is performed three times. Blank determination is carried out by pipetting 20 mL of indigocarmine solution into an Erlenmeyer flask and adding distilled water. Titrate with 0.01 N KMnO_4 solution until the endpoint is indicated by the solution turning pale pink.

Data Analysis

The data obtained in the study of determining tannin content in the ethanol extract of water guava (*Syzygium aqueum*) leaves were derived from the permanganometric method. The tannin content was calculated using the permanganometric calculation formula. The results obtained were then presented in the form of tables.

Formula for Determination of Tannin Content

$$\text{Tannin content (mg/mL)} = \frac{VKMnO_4 \times NKMnO_4 \times EW \times 1000}{V_{\text{sampel}}}$$

Description:

$VKMnO_4$ = Volume of KMnO_4 solution used (mL)

$NKMnO_4$ = Normality of KMnO_4 solution (N)

EW = Equivalent weight

1000 = Conversion factor from grams to milligrams

V_{sampel} = Volume of the sample analyzed (mL)

RESULTS & DISCUSSION

The permanganometric method is a redox titration carried out based on the reaction of potassium permanganate (KMnO_4). This titration involves two stages, namely the titration of the analyte with potassium permanganate solution and the subsequent standardization of potassium permanganate using sodium oxalate solution. The titration process involves volumetric manipulation to prepare the analyte solution.

The initial stage was a qualitative analysis to confirm the presence of tannins in the sample. In addition, a quantitative analysis was carried out to accurately determine the tannin content.

Table 1. Qualitative Test

Name	Reagent	Color Change	Result
Water guava (<i>Syzygium aqueum</i>) leaf extract	FeCl_3 Solution	Greenish Black	Positive for Tannins (+)

Based on Table 1, it is shown that before conducting quantitative analysis of tannin content, the samples were first analyzed using a qualitative test to identify the presence of tannin compounds. The determination of tannin content was carried out to measure the concentration of tannin compounds in water guava (*Syzygium aqueum*) leaves (Rizky Amelia, 2015).

Table 2. Quantitative Test

Name	Replica	Sample Volume (mL)	V. KMnO_4 (mL)	N. KMnO_4	EW (mg/meq)	V. Sample	Tannin Content (mg/mL)	Content (%)
Water guava leaf extract	1	10	33	0,01	85	10	2,805	28,05
	2	10	32	0,01	85	10	2,72	27,2
	3	10	33,5	0,01	85	10	2,8475	28,475
Total average tannin content								27,91%

Based on Table 2 above, the quantitative test for the determination of tannin compounds using standardized KMnO_4 solution was carried out three times, resulting in an average tannin content value of 27.91% in the ethanol extract of water guava (*Syzygium aqueum*) leaves (Mulyani et al., 2022).

In this study, the determination of tannin content was carried out in two stages, namely qualitative and quantitative tests. The qualitative test was conducted as an initial step to confirm the presence of tannin compounds in the water guava (*Syzygium aqueum*) leaf samples. The indication of tannin compounds was shown by the color change in the sample from greenish-black to yellowish-golden, which demonstrates the reaction between tannin compounds and 0.01 N potassium permanganate (KMnO_4) solution. Subsequently, the quantitative test was performed to accurately determine the tannin content using the permanganometric method. In this test, the titration endpoint was indicated by a color change of the solution from yellow to golden yellow, as a result of the reaction between tannin compounds and indigocarmine solution as the indicator.

In the determination stage of tannin content in water guava (*Syzygium aqueum*) leaves using permanganometric titration, 1.5 g of ethanol extract of water guava leaves (*Syzygium aqueum*) was weighed, placed into a 100 mL beaker, and 50 mL of distilled water was added. The mixture was then heated at a temperature of 40–60 °C for 30 minutes. After cooling, the solution was filtered into a 250 mL volumetric flask, and distilled water was added up to the calibration mark. From this solution, 25 mL was taken and placed into an Erlenmeyer flask, followed by the addition of 20 mL of indigocarmine solution. The titration was then carried out using 0.01 N KMnO_4 solution until the solution produced a light purple/pale pink color that persisted. This process was repeated three times to obtain results with the tannin content value at the titration endpoint for the water guava leaf (*Syzygium aqueum*) sample. From this study, the tannin content in water guava leaves (*Syzygium aqueum*) was found to be 27.91%.

Based on the analysis using the permanganometric titration method, the tannin content obtained from three repetitions was as follows: in the first trial, with a volume of 33 mL, the tannin content was 2.805 mg/mL, equivalent to 28.05%. In the second trial, with a volume of 32 mL, the tannin content was 2.720 mg/mL or 27.2%, while in the third trial, with a volume of 33.5 mL, the

result was 2.8475 mg/mL or 28.475%. From these three data sets, the average tannin content was 2.7908 mg/mL or 27.91%. According to the literature, tannin content is considered high when it exceeds 10% and low when it is below 10% (Siqueira et al., 2012). Therefore, the tannin compounds obtained in this study of water guava (*Syzygium aqueum*) leaves fall into the very high category, since the tannin content obtained was greater than 10%. Environmental factors such as climate, freshness of the samples at the time of collection, and the methods used can also influence the results obtained.

The main difference between this study and previous research lies in the sample object, the analytical method used, and the tannin content obtained. The previous study by Mulyani et al. (2022) investigated tannin levels in pagoda leaves (*Clerodendrum paniculatum*) using two methods, namely visible spectrophotometry and permanganometric titration. The results showed relatively low tannin levels, ranging from about 4.14–7.66% with spectrophotometry and 0.443% with permanganometry. Meanwhile, this study focused on water guava (*Syzygium aqueum*) leaves using only the permanganometric method. The titration results showed a tannin content of 27.91%, which is significantly higher compared to the previous study.

CONCLUSION

Based on the results of this study, it can be concluded that the ethanol extract of water guava (*Syzygium aqueum*) leaves was proven to contain tannin compounds. The tannin content of the water guava leaves, determined through permanganometric titration until the endpoint, was found to be 27.91%. This tannin level falls into the very high category, as it exceeds 10%.

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