

Phytochemical screening analysis of Guava leaf extract (*Psidium guajava L.*) against the content of Saponins, Tannins, and Flavonoids

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ABSTRACT

Guava is a plant with many benefits, especially in the leaves. There are many chemical compounds in guava leaves (*Psidium guajava L.*), such as flavonoids, tannins, and saponins. Traditionally, Guava leaves have been used for a long time (*Psidium guajava L.*) as medicine to stop diarrhea, with its availability as an herbal product widely marketed. The use of guava leaves as traditional medicine is made by decoction the leaves and extracting them to obtain chemical compounds, namely Tannins found in the leaves. In addition to decoction, guava leaf extract can be obtained by maceration. Research that has been conducted aims to determine the content of saponin compounds, tannins and flavonoids in guava leaves (*Psidium guajava L.*) using two extraction methods, namely maceration and decoction. The maceration method uses a technical ethanol solvent (96%), and decoction uses a water solvent. Then phytochemical screening is tested based on the color reagent of the compound. Based on the results of research that has been carried out, positive guava leaf extract (*Psidium guajava L.*) contains saponin compounds characterized by the formation of stable foam, tannins in the presence of a bluish-black or green color, and flavonoids in the presence of a dark red color.

Keywords:

Guava Leaf (*Psidium guajava L.*); Tannins; Saponins; Flavonoids; Extraction.

Introduction

Guava leaves (*Psidium guajava L.*) have an elliptical, slender, or oval round shape and a blunt or pointed tip. The Guava leaf plant (*Psidium guajava L.*) is one of the traditional medicinal plants that can be used to treat diarrhea or loose stools. Guava leaves are easily obtained plants because they are widely available in Indonesia. Based on the results of phytochemical testing that has been done, it shows that dry leaves positively contain steroid compounds, saponins, phenols, and tannins. Meanwhile, positive fresh leaves contain alkaloid compounds, steroids, saponins, phenols, and tannins (Simbolon et al., 2021). The results of other studies also showed that qualitative phytochemical testing of guava leaf extract contains antibacterial compounds such as saponins, tannins, and flavonoids (Handarni et al., 2020). The leaf part of this plant has a higher effectiveness as an anti-diarrheal due to the active components that are widely found in the leaves, such as tannins, flavonoids, and saponins (Ujan et al., 2019).

Tannins in the health sector have pharmacological activities such as anti-diarrheal, anti-oxidant, anti-bacterial, and astringent. Tannins as antibacterials have the same ability as phenolic compounds to precipitate bacterial proteins and are interrelated with antidiarrheal activity, namely in diarrhea caused by inflammation from bacteria (Sunani & Hendriani, 2023). Guava leaf extract (*Psidium guajava L.*) can be given as a potential antidiarrheal in children due to the presence of tannin compounds (Faulinza, 2022). In addition to tannins, flavonoids, and saponins in guava leaves, there are also phenols, triterpenes, essential oils, carotenoids, lectins, vitamins, fiber, and fatty acids (Adamu, 2021). The results of phytochemical screening show that

flavonoid compounds, tannins, saponins, alkaloids, steroids, and terpenoids are useful as antibacterial active compounds (Widhowati et al., 2022).

Flavonoids are the most abundant polyphenolic compounds found in plants (Maleki et al., 2019). Flavonoids are also found in vegetables, nuts, tea plants, dark chocolate. Flavonoids have anti-inflammatory potential and also have a profound impact on several immune cells and body immunity through various important processes in inflammation (Sanjaya et al., 2023).

Saponins are a class of secondary metabolite compounds found in plants. Saponins are also phytochemical compounds characterized by the ability to form foam with polycyclic aglycone content bound to one or more sugars (Suleman et al., 2022). Saponins have various biological properties, such as hemolytic ability, antimollusk, antiviral activity, cytotoxic or anticancer activity, hypocholesterolemia effects, and antiprotozoa (Purnamaningsih et al., 2017).

The results of research that other researchers have carried out previously showed that the phytochemical test results of guava leaf extract against flavonoid compounds, tannins, saponins, and alkaloids were positive, where the antibacterial activity of guava leaf extract was shown to be inhibitory against *Escherichia coli* bacteria (Niken et al., 2022)(Widiastuti et al., 2023). The chemical structure of these compounds can be seen in the following figure 1.

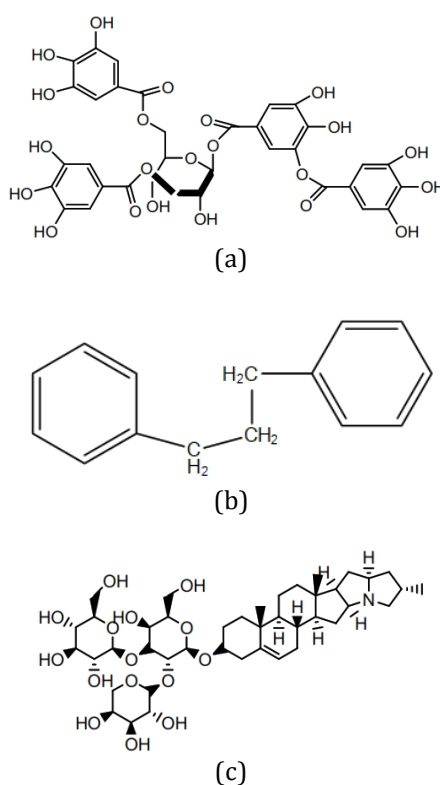


Figure 1. Compound Structure (a) Tannins, (b) Flavonoids, and (c) Saponins (Noer et al., 2018)

Tannins, flavonoids, and saponin compounds can be obtained by carrying out the extraction process using solvents. Extraction is the process of separating compounds from simplisia using appropriate solvents. Extraction is also an event of solute transfer (solute) between two solvents that do not mix (Hanani, 2017). Extraction is done by maceration and decoction. Maceration is one method of separating compounds by soaking them in organic solvents at certain temperatures (Karina et al., 2016). During the material soaking process, there will be a breakdown of the cell wall and cell membrane caused by the pressure difference between the outside of the cell and the inside of the cell, so that secondary metabolites in the cytoplasm will break and dissolve in the organic solvent used (Novitasari & Putri, 2016). The advantage of the maceration method is that the extraction process is simple and practical,

carried out by soaking simplisia under continuous cold conditions to attract the desired compound. However, the disadvantages of this maceration method are the length of the extraction time and the amount of solvent needed in the extraction process (Putra et al., 2014). Decoction is the process of heating food ingredients in a decoction liquid. In decoction, food ingredients can be put into water before or after decoction water. The heating process can cause an increase or decrease in the levels of chemical compounds contained in the sample. The length of the decoction time is related to the time or opportunity that the solvent has to come into contact with the boiled material (Dhyanaputri et al., 2022). The purpose of this study was to identify tannins, flavonoids, and saponins using two methods, namely maceration using ethanol and decoction with water solvent

Methods

This research was conducted at the Chemistry Laboratory of the Chemistry Education Study Program, Faculty of Teacher Training and Education, Syiah Kuala Darussalam University. This study used laboratory-scale experimental methods. The research method used in this study is a qualitative descriptive method to determine the results of phytochemical screening contained in guava leaves (*Psidium guajava* L.). Phytochemical screening includes tests for flavonoids, tannins, and saponins. The extraction technique is carried out using two extraction methods, namely maceration and decoction. The maceration method uses technical ethanol solvent (96%), and decoction uses water solvent. Then phytochemical screening is tested based on the color reagent of the compound.

Tools and Materials

Tool

The tools used are 6 test tubes (pyrex), 6 droppers, 1 spatula, 3 beakers 50 mL (pyrex), 1 beaker 500 mL (pyrex), 1 measuring cup 100 mL (pyrex), 1 glass, 1 analytical scale (HWH TYPE DJ 602 c atau 1002 c), 1 scissors, and 1 stirring rod.

Material

The ingredients used in this study were 300 grams of guava leaves for maceration and decoction, 100 ml of technical ethanol 96% p.a (Merck), 50 ml of aquadest, 10 ml concentrated HCl (Merck), 20 ml of FeCl₃ (1%), 1 gram of Mg powder, Whatman filter paper, and aluminum foil.

Procedure

Sample Preparation

Fresh guava leaves are washed using running water to remove dirt that is still attached. As much as 1 kg of guava leaves is washed, cut into smaller pieces, and then mashed without using solvents by pounding or blending.

Sample Extraction

Sample extraction using the maceration method is carried out by preparing leaf extract. Guava leaves are macerated with 96% ethanol solvent. Soaking is carried out for 6 hours at a time stirring. Maceration is separated by filter using a glass funnel held in a place by whatman filter paper to separate the pulp from the residue. The remaining residue in the first soaking is macerated again using 96% ethanol. All extraction results that have been obtained are collected and stored for 24 hours, then filtered again and evaporated until a thick guava leaf extract is obtained (Abebe et al., 2022).

The extraction method is by decoction; the mashed sample is put into a pot filled with water, as much as 500 mL, and cooked on a medium-burning stove for 15 minutes. The results

of the decoction are cooled, and then the boiled extraction water is separated from the pulp of the guava leaf samples.

Phytochemical Screening Qualitative Test

To identify tannin compounds, plant samples have been extracted with ethanol or boiled. Then, as much as 1 ml of solution is transferred into a test tube and 2-3 drops of 1% FeCl_3 solution are added. Meanwhile, to identify saponin compounds as much as 2 mL of plant samples that have been extracted with ethanol or boiled are put into a test tube, plus distilled water so that all footage is submerged, boiled for 2-3 minutes, and then cooled, then shaken vigorously. For flavonoid tests, 2 mL of plant samples extracted with ethanol or boiled are heated for five minutes in a test tube. Next, add a few drops of concentrated HCl. Then 0.2 g of Mg powder was added (Rubianti et al., 2022) (Peasari et al., 2018).

The positive result of tannin formation is characterized by the presence of bluish-black or green color which indicates the presence of phenol compounds in the extraction material. Then, if the extract contains saponin compounds, it can be seen from the formation of foam or foam that is stable for 30 minutes and does not disappear by addition of 1 drop of HCl 2 N. If there are flavonoids in the extract, the formation of red, yellow, or orange colors in the amyl alcohol layer (Tambalean et al., 2023).

Results and Discussions

The initial process of research is to extract samples by maceration and decoction. Extraction by maceration used 96% technical ethanol solvent. Maceration is the immersion of a sample that has been finely blended with ethanol solvent and allowed to stand for 24 hours. After 24 hours, the extract is separated from the leaf pulp by filtering to obtain the extract to be analyzed. The extract obtained is brown.

The extraction method is by decoction water solvents are used, water is used because water is an easily obtained solvent and is often used when decoction leaves to be used as natural medicine. This decoction is done in about 15 minutes, and the sample is not mashed. After decoction, it is cooled then the extract and boiled leaves are filtered. The reaction results obtained for maceration and decoction extraction methods are 20 mL and 250 mL, respectively. The extract of the decoction is dark brown. The following is a picture of the filtered extract obtained from both methods.

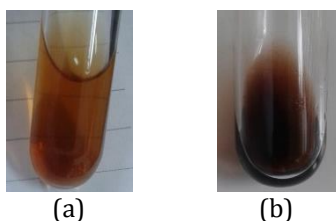


Figure 2. (a) Maceration extract, (b) Decoction extract

Phytochemical Screening Qualitative Test

The samples underwent qualitative phytochemical screening tests on tannins, saponins, and flavonoids. Based on the results of tannin tests, the reaction between polyphenols and FeCl_3 forms a variety of colors that indicate the presence of complex compounds depending on the substituents bound to polyphenols. The analysis results obtained guava leaf extract positively containing tannins with a change in color to blue or blackish green. Furthermore, the saponin test results form a stable foam after shaking vigorously. Then, the last flavonoid test with the results obtained is the occurrence of a change in color to red. These changes show that guava leaf extract contains flavonoids. The results of the analysis can be seen in Table 1.

Table 1. Phytochemical screening qualitative positive test



Uji	Observations	Extraction Method	
		Maceration	Decoction
Tannins	Bluish-black or green color	+	+
Saponins	Formation of stable foam	+	+
Flavonoid	Dark red color	+	+

Based on the results of the research that has been done and the positive tests between maceration extract and guava leaf water decoction, different results were obtained. This difference is found in the formation of positive colors from each test performed. This is because at the beginning of the extraction process, all compounds in the leaves will be extracted and mixed with solvents. In addition, the difference is due to the influence of the temperature of the heating process. The length of the heating time causes the yield to break down. The solubility of the extract in the material runs slowly in proportion to the increase in time, but after reaching the optimal time, the number of components taken from the material will decrease, causing damage, and the yield produced will decrease. The components in the extract are damaged because they are not resistant to heat (Koesnadi et al., 2021).

Phytochemical Screening Tannins

The tannin test is carried out by adding a 1% solution of iron (III) chloride (FeCl_3). It is estimated that this solution reacts with one of the hydroxyl groups present in the tannin compound. The results of the reaction obtained cause color and the formation of deposits. FeCl_3 reagents are widely used to identify phenol compounds, including tannins. The test results obtained from the two extraction methods used are the formation of blue-black or green, as shown in the table 2 tannins test result.

Tabel 2. Tannins test result

Test	Observations	Test Result
Tannin	 Maceration: Bluish-black or green color	(+)
	 Decoction: Bluish-black or green color	(+)

Tannins are divided into two groups and each each giving a different color reaction to 1% FeCl_3 the hydrolyzed tannins group will produce a blue-black color and condensed tannins will produce a blackish-green color. At the time of addition, it is estimated that FeCl_3 reacts with one of the hydroxyl groups in tannins compounds, and FeCl_3 is a compound containing metals so that when it reacts with tannins, Fe^{3+} will be reduced to Fe^{2+} and will form chelate complex compounds with tannins (Soamole et al., 2018). The result of the reaction is what finally causes color. As shown in the table 2 of tannin test results with the extraction method used, the formation of a bluish-black or green color.

The extraction in guava leaf extract dramatically affects the obtained tannin content. An extraction process that is too long will result in damage to the tannins content and the extraction process that is too short will result in a less than optimal tannins content. The solubility of the active substance extracted will produce an optimal extract with increasing

temperatures and the length of extraction time used. However, increasing the temperature and extraction time used needs to be considered because temperatures that are too high and long extraction times can produce low yields (Ibrahim et al., 2015).

Saponins

The chemical structure of saponin is a type of glycoside consisting of a glycone and an aglycone. The glycone part involves groups of sugars such as glucose, fructose, and other types of sugar. Meanwhile, the aglycone part is sapogenin. As explained by Nurzaman (2018), its amphiphilic nature makes natural ingredients containing saponins function like surfactants. Saponins themselves are glycosides with aglycones in the form of steroids and triterpenoids. Despite having various glycosyl groups attached to the C3 position, some saponins have two sugar chains attached to the C3 and C17 positions. This saponin structure gives it properties similar to soap or detergent, so it is often referred to as a natural surfactant (Hawley & Hawley, 2004). Pictures of saponin structures in the form of steroids and triterpenoids can be seen in Figure 3.

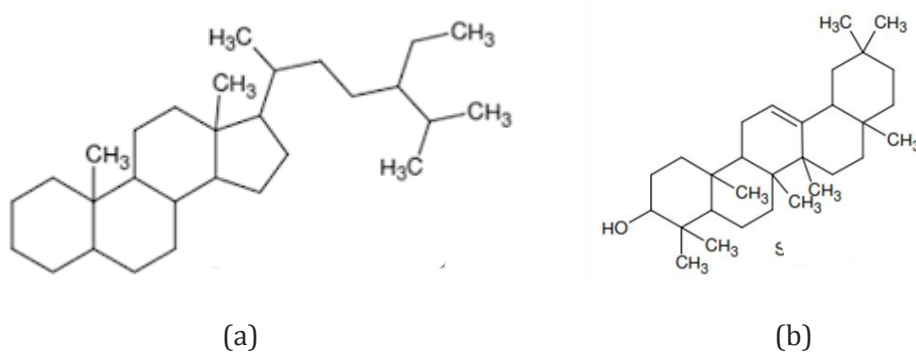




Figure 3. (a) Steroids; (b) Triterpenoids

Tabel 3. Saponin test result

Test	Observations	Test Result
Saponin	 Maceration: Formation of stable foam	(+)
	 Decoction: Formation of stable foam	(+)

Steroidal saponins are composed of a steroid core (C27) with a carbohydrate molecule (Hostettmann, 1995) and, if hydrolyzed, produce an aglycone known as sapogenin. Steroid saponins are mainly found in monocotyledonous plants such as the sansevieria group (Agavaceae), gadung (Dioscoreaceae), and flowering plants (Liliaceae). Triterpenoid saponins are composed of a triterpenoid core with a hydrolyzed carbohydrate compound to produce an aglycone known as sapogenin. Triterpenoid saponins are found in many dicotyledonous plants, such as legumes (legumes), areca nuts (Araliaceae), and caryophyllaceae. Several research results have shown the role of triperpenoid saponins as natural defense compounds in plants (Purnamaningsih et al., 2017). The result of that reaction finally causes foam in the saponin test, as shown in Table 3. Saponins are substances characterized by the presence of both hydrophilic and hydrophobic groups (Wardana, 2016).

Saponins, when shaken, form foam due to the presence of hydrophobes will bind to air while hydrophilic groups that bind to water. In the micelle structure, non-polar groups face inward while polar groups face outward. This situation makes foam form (simaremare, 2014).

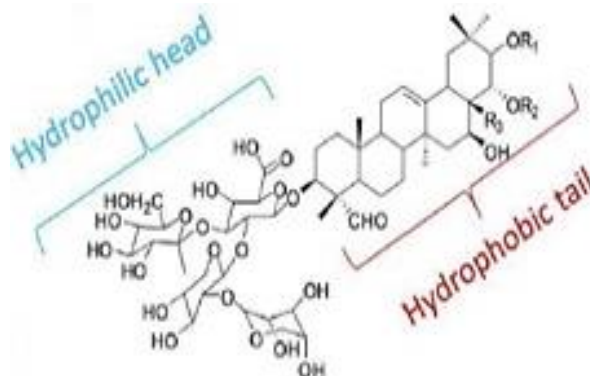




Figure 4. Hydrophilic and hydrophobic groups on saponin structures

Flavonoids

The test results in guava leaf extract showed a change in color to the sample tested, namely dark red color; this is due to the addition of magnesium powder and hydrochloric acid in flavonoid testing. The result of the reaction is what characterizes the presence of flavonoid compounds. Table 4 show Flavonoid test result.

Table 4. Flavonoid test result

Test	Observations	Test Result
Flavonoid	 Maceration: dark red color	(+)
	 Decoction: dark red color	(+)

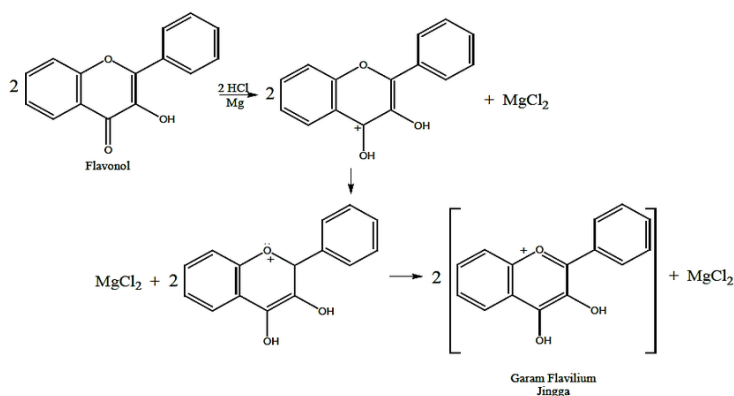


Figure 5. Reaction of flavonoids with HCl and Mg metals

The addition of magnesium powder and hydrochloric acid to flavonoid testing, which induces a red color reaction characterizing the presence of flavonoids, will lead to the reduction of existing flavonoid compounds (Robinson, 1995). Moreover, the function of concentrated Mg

and HCl metals in this test reduce the benzopyron core contained in the flavonoid structure so that the color changes to dark red or orange. If in a plant extract, there are flavonoid compounds, flavilium salts will form when adding red or orange Mg and HCl (Setyowati et al., 2022).

Conclusion

Based on the results of phytochemical screening tests on guava leaf extract (*Psidium guajava* L.) that have been carried out, it can be concluded that the extraction results using maceration methods and guava leaf decoction (*Psidium guajava* L.) show a bluish-black or green color in tannin tests, positive saponin tests for stable foam formation, and positive flavonoid tests give a dark red color.

Acknowledgments

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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