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Identification of Secondary Metabolites in Guava Leaf Extract (*Psidium guajava* L.)

Original Article

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Abstract:
Guava leaves (*Psidium guajava* L.) are known for their significant potential as a natural therapy for diabetes due to their bioactive compound content. This study sought to determine the secondary metabolite chemicals present in the ethanolic extract of guava leaves sourced from Tegal, Central Java, via phytochemical analysis. The extraction process employed the maceration method, utilizing 96% ethanol as the solvent. The screening of phytochemicals includes assays for tannins, saponins, steroids/terpenoids, alkaloids, and flavonoids. However, the steroid test yielded a negative result. These findings indicate that guava leaves are a rich source of active compounds, particularly flavonoids, alkaloids, and terpenoids, which are believed to play a role in lowering blood glucose levels.

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INTRODUCTION

Guava is a plant native to Central America, which then spread and expanded to Southeast Asia and to Indonesia via Thailand (Fadhilah et al., 2018). This plant thrives in tropical and subtropical regions (Mahalaksmi et al., 2024). Guava can grow in both lowland and highland areas up to an elevation of 1,200 meters above sea level. It can be cultivated in well-drained to clay soils, particularly in open locations with adequate water availability (Muhlisah, 2007).

The guava plant exhibits morphological traits such as a taproot and a stem with a smooth surface and thin, easily removable bark (Rahma et al., 2023). The guava plant has young, quadrangular stems, while the older stems are hard, woody, brown, and can grow up to 9 meters tall. The leaves are simple, oblong in shape, and emit a distinctive aroma when crushed. Guava fruit is a single-seeded fruit with thin skin and a surface ranging from smooth to rough (Fadhilah et al., 2018).

Based on 2017 statistics from Badan Pusat Statistik (BPS), guava (*Psidium guajava* L.) is one of the top ten fruit production commodities in Indonesia. Overall, the output of fruits and vegetables demonstrated a positive trajectory that year when compared to 2016. With a manufacturing output of 200,495 tons, there exists considerable potential to improve the exploitation of various plant parts, particularly guava leaves, on a wider scale (Handarni et al., 2020).

One of the many potential uses for guava leaves is as an antidiabetic medication.

Figure 1. Fresh Guava Leaves

Psidium guajava's capacity to lower insulin resistance and enhance pancreatic function supports its promise as a natural diabetes treatment. Guava functions by obstructing the actions of the enzymes α-amylase and α-glucosidase, which facilitate carbohydrate degradation. Among the roughly 300 bioactive constituents present, five principal compounds —Morin, Apigenin, Quercetin, (+)-Catechin, and Psiguadial D—function as enzyme inhibitors to regulate blood glucose levels (Fadhilah et al., 2018).

Guava leaves contain chemical compounds useful for medicinal purposes, including alkaloids, flavonoids, and tannins (Yanuarty et al., 2021). Additionally, guava leaves are known to contain polyphenols that act as antioxidants (Gunata, 2021). According to previous research, the flavonoids in guava leaves have a very high quercetin content. Quercetin is believed to induce glucose uptake by liver cells, thereby lowering blood glucose levels (Gusprativi et al., 2019).

In order to determine the secondary metabolites found in the crude extract of Guava Leaf (*Psidium guajava* L.), phytochemical screening is crucial since the concentration and composition of secondary metabolites can be affected by the environmental and geographic circumstances in which the plant develops. In order to characterize the phytochemical profile of Guava Leaf (*Psidium guajava* L.) collected from Tegal, Central Java, and to provide initial information about the bioactive compounds that may contribute to its potential pharmacological activities, secondary metabolites must be identified. Thus, the purpose of this work was to do a phytochemical screening of the crude extract by examining the presence of alkaloids, flavonoids, steroids/terpenoids, tannins, and saponins.

METHODS

Preparation of Raw Materials

Guava leaf samples were collected from Tegal, Central Java. Fresh guava leaves were washed under running water to remove any remaining dirt, then dried until they were dry and brittle. The leaves were ground and sieved using a 40-mesh sieve. The moisture content of the crude drug powder was tested at a temperature of 105°C.

Extract Preparation

Extraction of the guava leaf crude powder was performed using the maceration method with 96% ethanol as the solvent. The solvent-to-material ratio is 8:1. The required amount of dry leaf powder is 300 grams, and 2.4 liters of solvent are used in the first extraction. The extraction process lasts for 3 × 24 hours, with the mixture stirred occasionally each day. After maceration, the mixture is filtered using filter paper to separate the maceration product from the residue. The resulting residue is then subjected to a second maceration using 2.4 liters of fresh solvent without adding any additional plant material. Next, the extraction products from the first and second extraction processes are combined and concentrated using a rotary evaporator at a temperature of 65°C.

Flavonoid Content Test

A 0.5-gram sample of the extract was taken, mixed with 5 mL of ethanol, and placed in a test tube. The mixture was heated for approximately 5 minutes, and 10 drops of concentrated HCl and 0.2 grams of magnesium powder were added. A positive result was indicated by the appearance of a reddish-black, orange, or yellow color (Septianingsih et al., 2020).

Test for Steroids/Terpenoids

Dissolve 0.5 grams of the extract sample in chloroform, then slowly add 2 mL of Liebermann-Burschard reagent (anhydrous acetic acid–H_2SO_4) along the wall of the test tube, and observe the color change that occurs. The solution is gently shaken and allowed to stand for several minutes. The presence of steroids is indicated by the formation of a green to bluish color, while terpenoids are indicated by the formation of a red to purplish-

brown color (Andry & Sastra Winata, 2023).

Saponin Content Test

A 0.5-gram sample of the extract is placed in a test tube and 10 mL of hot distilled water is added. The test tube is vigorously shaken for 60 seconds. The mixture is then allowed to stand for 10 minutes and observed. A positive result is indicated by the formation of stable foam that does not easily dissipate (Ginting & Andry, 2023).

Tannin Content Test

A 0.5-gram sample of the extract was mixed with 10 mL of hot water and placed in a test tube. Two to three drops of a 10% $FeCl_3$ solution were added to the test tube. A positive result is indicated if the solution turns a bluish-black or greenish color (Nasution et al., 2023).

Alkaloid Content Test

A 0.1-gram sample of the extract was placed in a test tube and 5 drops of 2 N HCl were added. Wagner's reagent was added to the tube; a positive result is indicated by the formation of a reddish-brown precipitate (Susanti et al., 2024).

RESULT AND DISCUSSION

Moisture Content

A moisture content test was conducted to determine the moisture content in the crude drug powder to prevent the growth of microorganisms that could reduce the quality of the crude drug. According to the test findings, the powdered crude extract of guava leaves has a moisture content of 2.45%. This indicates that the medicinal raw material meets the quality standard for moisture content, which is no more than 10% (Kemenkes RI, 2017). Thus, *Psidium guajava* leaves havea nearly equivalent level of safety regarding the risk of fungal or microbial growth. This suggests that the quality and stability of the active compounds can be maintained during storage.

Extract Yield

Extraction in this study was performed using the maceration method with 96% ethanolas the solvent. This method was chosen because it is simple and prevents damage to heat sensitive compounds (Katuuk et al., 2018). Ethanol was chosen as the solvent due to its semi polarnature, whichallowsit to optimallyextractboth polarand non-polaractivecompounds(Arsyad etal., 2023). Theextractionprocess yielded a thick extract weighing 92.2 grams with a viscous consistency; it was dark brown in color and had a distinctive aroma similar to that of the leaves, with an extraction yield of 30.74%. The resulting guava leaf extract meets quality standards. According to the Kemenkse RI (2017), a good yield for concentrated *Psidium guajava* leaf extract is no less than 12.3%. A higher extraction yield indicates that more extract was produced. Determining the extraction yield of a sample is important for knowing how much extract was successfully obtained during the extraction process (Saerang et al., 2023).

Phytochemical Screening

Phytochemical identification was performed qualitatively using color reaction methods to detect the presence of phenolic compounds, flavonoids, steroids, terpenoids, saponins, tannins, and alkaloids. The results of the phytochemical screening of guava leaf extract are shown in Table 1.

Based on the results of the phytochemical screening in Table 1, the ethanol extract of guava leaves was found to contain five active compounds: flavonoids, terpenoids, saponins, tannins, and alkaloids. These results are consistent with research conducted by Biadi et al., (2026). That study explained that the ethanol extract of guava leaves contains alkaloids, tannins, saponins, terpenoids, and flavonoids. This indicates that guava leaves have potential as a source of active compounds. Additionally, a research by Wardani et al., (2024) showed that an ethanol extract of guava leaves contained flavonoids, specifically quercetin. The guava leaf ethanol

Table 1. Results of Phytochemical Screening of *Psidium guajava* Extract

Secondary Metabolites	Reagents	Results
Flavonoids	HCl+ Magnesium Powder	+
Steroids	H_2SO_4	-
Terpenoids	H_2SO_4	+
Saponins	H_2O	+
Tannins	10% $FeCl_3$	+
Alkaloids	2N HCl + Wagner's Reagent	+

Note:
(+) : Positive result, indicating the presence of secondary metabolites
(-) : Negative result, indicating the absence of secondary metabolites

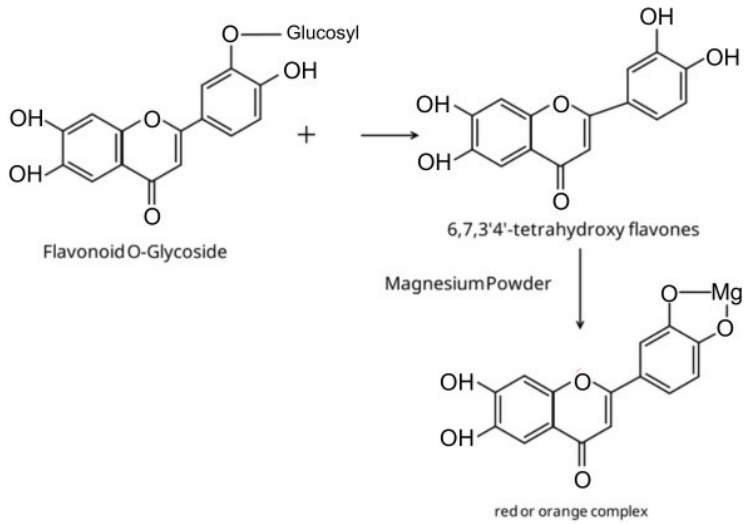

Figure 2. Flavilium Salt Formation Reaction

extract's Rf value in that investigation was determined to be 0.68, which is the same as the quercetin standard's Rf value of 0.68. Furthermore, UV-Vis spectrophotometry was used to quantify the quercetin concentration, which came out to be 2.87 ± 1.59%.

The phytochemical screening results for flavonoids in the guava leaf extract showed a positive result with the formation of a reddish-black solution after the addition of concentrated HCl and magnesium powder. The purpose of adding magnesium and HCl was to reduce the benzopyrone ring present in the flavonoid structure, thereby forming a flavilium salt (Tandi et al., 2020). The reaction is shown in the figure 2.

In the terpenoid test using Liebermann-Burschard reagent (anhydrous acetic acid–H_2SO_4), a positive result was observed with the formation of a reddish-brown color. The color change in the terpenoid test occurs through several reaction steps. The process begins with the acetylation of a hydroxyl group by anhydrous acetic acid, forming a double bond. Next, the release of a hydrogen atom shifts the double bond, causing the compound to undergo resonance and form an electrophilic carbocation. The formation of the carbocation then triggers the release of a hydrogen atom along with its electron, which increases the length of the compound's conjugated system. This structural change produces a color ranging from red to brownish-orange (Sulasmi et al., 2018).

The saponin test yielded a positive result, indicated by the formation of foam after the addition of distilled water and shaking. The emergence of foam during the saponin test signifies the existence of glycosides capable of generating bubbles in water, which are then digested into glucose and several other substances (Nurliansyah et al., 2024). Glycosides are composed of a saccharide known as glyco and a non-sugar component referred to as aglycone. Glycosides that connect these two compounds are easily broken down by the influence of acids, bases, water, enzymes, and heat (Sulasmi et al., 2018). The saponin foaming reaction can be seen in the figure 3.

Tannins reacted favorably with 10% $FeCl_3$, producing a dark green solution. This can happen when tannins and $FeCl_3$ combine to create a compound with Fe^{3+} ions

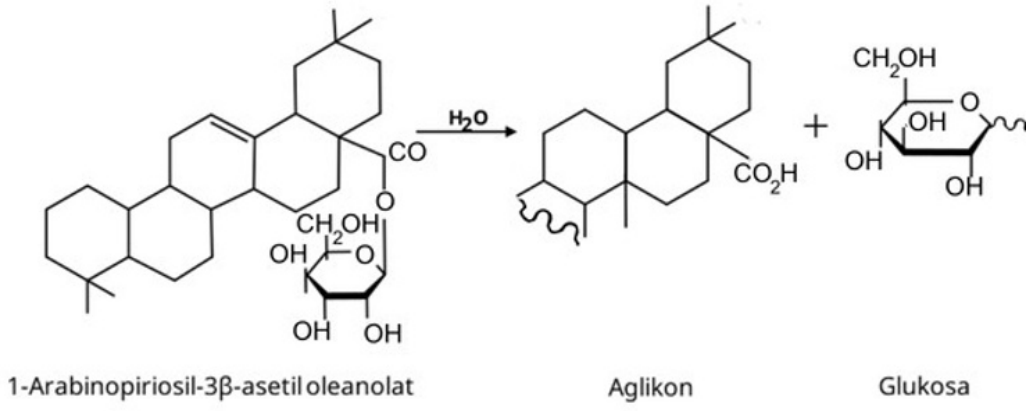

Figure 3. Saponin Foaming Reaction

(Septianingsih et al., 2020). Alkaloids were detected as positive, indicated by the appearance of a brown precipitate after reaction with Wagner's reagent. This precipitate is a potassium alkaloid formed by the interaction between the nitrogen in the alkaloid and the K+ metal ions in Wagner's reagent (Sulasmi et al., 2018). However, the steroid test yielded a negative result because no green to bluish color formed in the solution.

The presence of secondary metabolites identified in guava leaf extract has the potential to contribute to antidiabetic activity through various mechanisms. According to Abdulkadir et al., (2024), flavonoids specifically quercetin and catechin-containing flavonoids, such as those found in guava leaves can regenerate and protect pancreatic beta cells from damage and stimulate insulin release. Alkaloids are also known to regenerate damaged pancreatic β-cells, thereby increasing insulin secretion. This increase in insulin secretion results from the sympathomimetic effects of alkaloids (Arjadi & Mustofa, 2017; Khaerati et al., 2020). Terpenoids have been shown to improve cellular structure within the islets of Langerhans by increasing cell numbers, stimulating regeneration, and reducing apoptosis in various pancreatic cell types (Roy et al., 2024). Therefore, the presence of several classes of secondary metabolites in guava leaf extract may have synergistic effects on blood glucose control. However, the specific contributions of each compound and the predominant mechanisms of action still need to be confirmed through further research.

CONCLUSION

Based on the research conducted, the moisture content of guava leaf powder was found to be 2,45% and the yield of its extract was 30,74%. Phytochemical screening of the extract showed that the ethanolic extract of guava leaves (*Psidium guajava* L.) contains several important secondary metabolite compounds, such as flavonoids, terpenoids, saponins, tannins, and alkaloids. The screening for steroids yielded a negative result, as indicated by the absence of the characteristic color change during testing. These results are consistent with previous studies and confirm the potential of guava leaves as a source of bioactive compounds. The presence of these metabolites, particularly flavonoids and tannins, supports the potential of guava leaves in pharmacological applications, such as maintaining stable blood glucose levels.

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CONFLICT OF INTEREST

We declare that we don't have any conflict of interest.

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