BIOLOGY, MEDICINE, & NATURAL PRODUCT CHEMISTRY ISSN 2089-6514 (paper)
Volume 15, Number 1, April 2026 | Pages: 1103-1111 | DOI: 10.14421/biomedich.2026.151.1103-1111 ISSN 2540-9328 (online)

Phytochemical Identification of Secondary Metabolite Compounds in Leaf Extracts of *Sente* (*Alocasia macrorrhizos*), Guava (*Psidium guajava* L.), and Their Combination

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Manuscript received: 28 January 2026. Revision accepted: 03 June 2026, Published: 22 June 2026.

Abstract

The presence of secondary metabolites in plants plays an important role in their biological activities and supports the use of natural ingredients as candidates for phytopharmaceutical development. This study evaluated the phytochemical profile of ethanol extracts of *sente* leaves (*Alocasia macrorrhizos*) and guava leaves (*Psidium guajava* L.), as well as their combined extract. The extracts were obtained using maceration, followed by qualitative phytochemical screening, UV-Vis spectrophotometry, and FTIR analysis. Physicochemical parameters showed that the moisture content of sente and guava leaves was 4.251% and 4.617%, respectively, while the ash content was 0.97% for sente and 0.59% for guava leaves. The extraction yield of guava leaves (3.81%) was higher than that of *sente* leaves (2.59%). The results showed that *A. macrorrhizos* contained flavonoids, tannins, and triterpenoids, whereas *P. guajava* exhibited a more complete profile, including alkaloids, saponins, flavonoids, tannins, and triterpenoids. The combined extract yielded positive results for almost all tested metabolite groups. The UV-Vis spectra showed absorption peaks in the range of 321–369 nm, while the FTIR spectra revealed the presence of –OH, aliphatic C–H, aromatic C=C, and C–O functional groups. Overall, the findings indicate that both extracts and their combination have strong potential as sources of bioactive compounds and may be further developed for phytopharmaceutical applications.

Keywords: *Alocasia macrorrhizos*; *Psidium guajava* L.; phytochemistry; secondary metabolites; *spectrophotometer* UV-Vis; FTIR.

Abbreviations: FTIR, Fourier Transform Infrared Spectroscopy; UV–Vis, Ultraviolet–Visible Spectrophotometry; C=C, aromatic carbon–carbon double bond; C–H, aliphatic carbon–hydrogen bond; C–O, carbon–oxygen bond; –OH, hydroxyl group.

INTRODUCTION

Indonesia, as a country endowed with abundant floral wealth, features thousands of plant species utilized generationally as traditional medicines. The use of easily accessible medicinal plants serves as a relatively safer alternative treatment with lower risks of side effects compared to synthetic drugs, while also reflecting the preservation and practice of ancestral cultural heritage in utilizing natural resources. The success of a plant as medicine is largely determined by its chemical compounds secondary metabolites synthesized by plants as self-defense mechanisms that exhibit important biological activity in humans. These secondary metabolites have been widely employed as dyes, poisons, food flavorings, and pharmaceuticals, showing high diversity and classifiable into several groups of natural compounds, including saponins, steroids, tannins, flavonoids, and alkaloids (Whika *et al*., 2017).

According to Farid *et al*. (2023), secondary metabolites like phenols, terpenoids, and alkaloids from various plant extracts possess antioxidant, anti-inflammatory, antimicrobial, or antibacterial properties. Consequently, communities, particularly in Indonesia, utilize surrounding plants as affordable alternative treatments with minimal side effects. Two such plants are sente leaves (*Alocasia macrorrhizos*) and guava leaves (*Psidium guajava* L.).

Sente (*Alocasia macrorrhizos*), a plant from the Araceae family, is commonly found in tropical regions and consumed daily. While some communities use its corm as food, fewer recognize the potential of other parts, particularly the leaves. *Alocasia macrorrhizos* contains aloceradamide, lignanamide, piperidine, and isotriglochin, which show potential as diuretics, anticancer agents, antioxidants, and antimicrobials (Phom *et al*., 2020; Yuliana & Fatmawati, 2018). Despite its great potential, the use of sente leaves as a medicinal

raw material remains limited, making phytochemical identification crucial to reveal its pharmacological value.

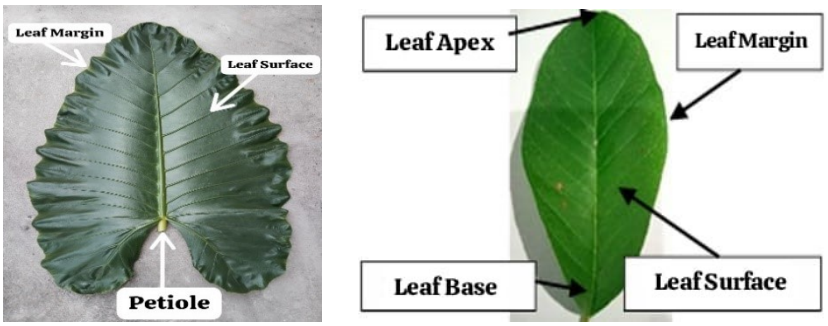
Unlike *sente*, guava leaves (*Psidium guajava* L.) have long been widely recognized and contain various compounds with antibacterial and antioxidant potential. Communities use guava leaves to treat diarrhea, dysentery, and other digestive issues. Guava leaves contain active compounds such as quercetin, polyphenols, quinones, saponins, alkaloids, flavonoids, and tannins, which act as antibacterials capable of inhibiting the growth of bacteria including *Staphylococcus aureus, Streptococcus* spp*., Salmonella typhi, Shigella dysenteriae,* and *Escherichia coli* (Girsang *et al*., 2020).

The first step in developing herbal medicines from natural sources is identifying secondary metabolites through phytochemical screening. Without this stage, scientific and measurable utilization of the plants cannot be achieved. This study aims to qualitatively identify secondary metabolite compounds in extracts of *sente* leaves and guava leaves. By identifying the phytochemicals in both, this research provides foundational information on the compounds present, particularly in sente and guava leaves, for future phytopharmaceutical development.

MATERIALS AND METHODS

Procedures

Sample Collection



(a) (b)

Figure 1. Figure 1. Fresh leaves of (a) sente (*Alocasia macrorrhizos*) and (b) guava (*Psidium guajava*) collected as raw materials for extract preparation.

Sente leaves and guava leaves were obtained from healthy plants of uniform age in the Lowokwaru area, Malang City. Leaves at various stages of maturity were manually picked from the middle part of the plant and then stored in separate containers. The samples were subsequently transported to the laboratory for sorting, cleaning, drying, and extract preparation to maintain consistency in material quality.

Preparation of Sente Leaf and Guava Leaf Samples-2

A total of 700 grams each of *sente* leaves and guava leaves were sorted, washed, and cut into small pieces before being dried in an oven at 65°C for 1–2 days. After drying, the leaves were ground using a blender and sieved to obtain fine simplicia powder.

Moisture Content Determination of Sente Leaf and Guava Leaf Samples-3

Prior to extraction, 1 gram of each *sente* leaf and guava leaf simplicia was weighed using an analytical balance to determine moisture content in order to ensure material quality and resistance to biological and microbial degradation (Sinaga et al., 2024). Measurements were carried out using an automatic moisture analyzer in accordance with BPOM standards, with a threshold of less than 10% and an ideal standard of 5%.

Ash Content Determination of Sente Leaf and Guava Leaf Samples-4

Ash content determination was performed by placing 1 gram (W1) of *sente* leaf and guava leaf simplicia into a porcelain crucible that had been previously weighed (W0). The simplicia was then heated using a muffle furnace at 400–600°C for 4–6 hours until white ash was formed. The obtained ash was then calculated using the following formula:

$$\% \text{ Total Ash Content} = (W2 - W0) / W1 \times 100\%$$

Notes:
W0 = Weight of ignited silicate crucible
W1 =Initial weight of simplicia sample
W2 = Weight of sample after ignition

Preparation of Ethanol Extract of Sente Leaves and Guava Leaves-5

Each 100 grams of *sente* leaf and guava leaf simplicia was macerated using 700 mL of 70% ethanol (a mixture

of 730 mL of 96% ethanol and 270 mL of distilled water), then shaken using a shaker for 2 hours/day and left to stand for 1–2 days in a place protected from light (Wahyuni et al., 2018). The filtrate was filtered, and the residue was remacerated with 300 mL of 70% ethanol, shaken for 1 hour, and left again for 1–2 days. All collected filtrates were then concentrated using a rotary evaporator at 70–80°C to obtain a thick extract.

Calculation of Extract Yield-6

Extract yield is one of the indicators used to measure extract quality. According to Haslindha (2022), yield values indicate the amount of bioactive compounds present in plants, and a high extract yield indicates a high content of dissolved substances in the raw material. The obtained extract was then weighed, and the yield was calculated using the following formula:

% Yield = (Weight of extract / Weight of sample) × 100%

Identification of Secondary Metabolite Compounds-7

Preparation of Sample Solutions. Each of the *sente* leaf and guava leaf extracts was weighed at 1 gram and then dissolved in distilled water to a final volume of 10 mL. For the combined sample solution, 0.5 gram each of sente leaf extract and guava leaf extract was weighed and dissolved in distilled water to a final volume of 10 mL

Alkaloid *Mayer.*Test tubes were prepared and labeled with the sample name and test name. Then, 10–20 drops of the sample solution were added into the test tube, followed by the addition of 3 drops of Mayer reagent into the extract. Alkaloids with Mayer reagent are indicated by the formation of a white or yellow precipitate (Djoko et al., 2020).

Alkaloid *Wagner.* Test tubes were prepared and labeled with the sample name and test name. Then, 10–20 drops of the sample solution were added into the test tube, followed by the addition of 3 drops of Wagner reagent into the extract. The formation of a dark brown or reddish-brown precipitate indicates the presence of alkaloids (Astryna et al., 2024).

Alkaloid *Dragendorff.* Test tubes were prepared and labeled with the sample name and test name. Then, 10–20 drops of the sample solution were added into the test tube, followed by the addition of 3 drops of Dragendorff reagent into the extract. A positive result indicating the presence of alkaloid compounds with Dragendorff reagent is marked by the formation of white/orange/yellow precipitate (Djoko et al., 2020).

Alkaloid *Bouchardat.* Test tubes were prepared and labeled with the sample name and test name. Then, 10–20 drops of the sample solution were added into the test tube, followed by the addition of 3 drops of Bouchardat reagent into the extract. A positive result indicating the presence of alkaloid compounds with Bouchardat reagent is marked by the formation of a brown precipitate (Djoko et al., 2020).

Flavanoid *Shinoda.* Test tubes were prepared and labeled with the sample name and test name. Then, 10–20 drops of the sample solution were added into the test tube, followed by the addition of 5 drops of ethanol and 3 drops of concentrated hydrochloric acid (HCl) into the extract. A small amount of Mg powder was then added. A positive result is indicated by orange color (flavone), pink (flavanol), red (2,3-dihydroflavanol), or purple (xanthone) (Ferdinan & Natasa, 2024).

Flavanoid *10% NaOH.* Test tubes were prepared and labeled with the sample name and test name. Then, 10–20 drops of the sample solution were added into the test tube, followed by the addition of 1 drop of 10% NaOH. A positive result is indicated by a yellowish-brown or reddish color (Amrullah et al., 2025).

Saponin. Test tubes were prepared and labeled with the sample name and test name. Then, 10–20 drops of the sample solution were added into the test tube, followed by the addition of 20 drops of previously heated distilled water. A positive result is indicated by the formation of foam with a height of 1–3 cm (Saras, 2020).

Tanin *Brymer.* Test tubes were prepared and labeled with the sample name and test name. Then, 10–20 drops of the sample solution were added into the test tube, followed by the addition of 1 drop of 10% $FeCl_3$. A positive result is indicated by a color change from green to bluish-black (Amrullah et al., 2025).

Tanin *Larutan Basa.* Test tubes were prepared and labeled with the sample name and test name. Then, 10–20 drops of the sample solution were added into the test tube, followed by the addition of 3 drops of 10% ammonium hydroxide (NH_4OH) along the wall of the tube. A positive result for tannin compounds is indicated by the appearance of a fluorescent yellow color (Amrullah et al., 2025).

Triterpenoid *Salkowski.* Test tubes were prepared and labeled with the sample name and test name. Then, 10–20 drops of the sample solution were added into the test tube, followed by the addition of 3 drops of concentrated H_2SO_4 along the wall of the tube. A positive result is indicated by the formation of a brown ring (Amrullah et al., 2025).

Extract Characterization-8

UV-Vis Spectrophotometry. UV-Vis Spectrophotometry characterization was carried out to determine the absorbance and wavelength of the extract samples.

FTIR. Characterization of guava leaf and sente leaf extracts was performed using FTIR (Fourier Transform Infrared) to identify potential biomolecules contained within them.

RESULTS AND DISCUSSION

Extraction Yield and Ash Content of *Alocasia macrorrhizos* and *Psidium guajava* L. Leaf

The extraction yield is a critical parameter in the extraction process that indicates the quantity of compounds successfully recovered from plant materials. This value reflects the solvent's efficiency in penetrating and extracting target compounds. A high yield suggests that the solvent is capable of dissolving a greater amount of active compounds from the material, whereas a low yield may result from solvent limitations, the extraction method employed, or the specific characteristics of the plant material, such as tissue structure and chemical composition (Parwati *et al*., 2023).

The results of this study showed that the extract yield of *Alocasia macrorrhizos* (*sente*) leaves was 2.59%, while the yield of *Psidium guajava* L. (Guava) leaves reached 3.81%. This difference indicates that guava leaves contain compounds that are more readily soluble in the chosen solvent compared to *sente* leaves. This finding is consistent with previous research stating that the content of secondary metabolites such as flavonoids, tannins, alkaloids, and phenolic compounds significantly influences the total extract produced (Rahmawati *et al*., 2025). The yield results suggest that guava leaves possess higher potential as a source of bioactive compounds than sente leaves. This condition highlights a more promising prospect for guava leaves in further development, particularly regarding biological activity studies and their application as phytopharmaceutical materials.

Based on the ash content analysis, the ash content obtained for *Alocasia macrorrhizos* was 0.97%, while *Psidium guajava* L. was 0.59%. These values indicate that both samples contain relatively low inorganic residues, categorizing them as having a high level of purity. Low ash content generally signifies minimal inorganic contaminants and superior quality of the herbal material (simplisia). The ash content values for both sente and guava leaves in this study are considerably low compared to other studies on medicinal plants, which have reported total ash content exceeding 10%. This confirms that both extracts maintain high purity levels and have the potential to be developed as phytopharmaceutical agents or sources of bioactive compounds (Simanjutak *et al*., 2025).

Phytochemical Screening Test

The purpose of phytochemical screening is to identify secondary metabolite compounds present in the extracts of *sente* leaves, guava leaves, and their combination. Phytochemical screening can be analyzed using various reagent tests, the results of which can be identified through color changes, precipitate formation, and other indicators. The results of phytochemical screening are presented in the table below.

Table 1. Phytochemical Screening Results of Sente Leaf Extract, Guava Leaf Extract, and Their Combination.

No	Secondary Metabolites	Reagents	Result Sente Leaf	Result Guava Leave	Result Combination
1	Alkaloid	Mayer	-	++	+
		Wagner	-	++	++
		Dragendorff	-	+	+
		Bouchardat	-	++	++
2	Flavonoid	Shinoda	++	++	++
		NaOH 10%	++	++	++
3	Saponin	Hot distilled water	-	++	+
4	Tanin	Brymers	++	++	++
		Alkaline solution	-	-	-
5	Triterpenoid	Salkowski	++	++	++

Notes:
++ : Indicates a strong positive reaction (Detected)
+ : Indicates a positive reaction with lower intensity (Detected)
− : Indicates a negative reaction (Not detected)

Table 1 shows the test results of *sente* leaf extract showed the presence of flavonoids, tannins, and triterpenoids, but were negative for alkaloids and saponins. The absence of alkaloids was indicated by the lack of precipitate formation in the Mayer, Wagner, Dragendorff, and Bouchardat tests, suggesting that their levels were very low or undetectable (Emilia et al., 2023). Flavonoids were identified through the Shinoda test (pink/red coloration) and the 10% NaOH test (yellowish-orange to reddish-brown coloration) due to the formation of phenolate salts (Sangkal et al., 2020). Saponins were considered negative because no stable foam was formed after heating and shaking (Aristyawan et al., 2024). Meanwhile, positive tannin results were indicated by a greenish-black color with Brymer reagent, and triterpenoids were confirmed by the appearance of a brown ring in the Salkowski test (Sandhori et al., 2025).

Guava leaf extract exhibited a complete phytochemical profile, containing all tested classes of compounds. The presence of alkaloids was confirmed by

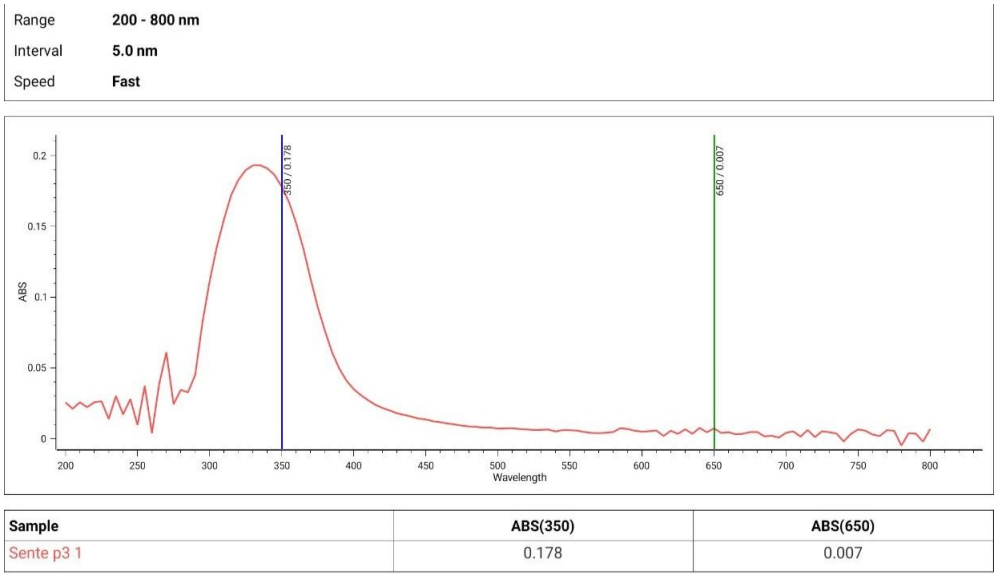
the formation of white precipitate in the Mayer test, reddish-brown precipitate in the Wagner test, and orange coloration in the Dragendorff and Bouchardat tests (Septiani, 2024). The presence of flavonoids (such as quercetin) was confirmed through the Shinoda and 10% NaOH tests, which play important roles as antioxidants and anti-inflammatory agents. Saponins were also detected through the formation of stable foam due to their surfactant properties, which are known to exhibit hemolytic effects and immunostimulatory potential (Saepudin et al., 2024).

Further analysis of guava leaves showed high tannin content, indicated by a greenish-black color change with Brymer reagent due to the complexation of Fe^{3+} ions with phenolic groups (Saepudin et al., 2024). These tannins function as astringent and antidiarrheal agents. In addition, the Salkowski test produced a brown ring, confirming the presence of nonpolar triterpenoids with pharmacological potential as anti-inflammatory, hepatoprotective, and anticancer agents (Septiani, 2024). The completeness of these bioactive compounds supports the wide traditional medicinal use of guava leaves.

The combination of sente (Alocasia macrorrhizos) leaf extract and guava (Psidium guajava L.) leaf extract exhibited a phytochemical profile that differed from those of the individual extracts. The most notable differences were observed in the alkaloid and saponin groups, which were not detected in the sente leaf extract but were positively identified in the combined extract. This finding indicates that the alkaloids and saponins originating from guava leaves remained detectable after mixing, although the reaction intensity of some alkaloid tests was reduced, likely due to a dilution effect. Meanwhile, flavonoids, tannins, and triterpenoids consistently showed strong positive reactions (++), suggesting that these compounds were present in relatively high concentrations and remained stable following the combination process. The combined extract demonstrated positive results for nearly all tested phytochemical parameters, including alkaloids, which formed insoluble complexes with classical alkaloid reagents (Harahap, 2023). Flavonoids were identified through the formation of colored complexes in the presence of magnesium and acid, as well as color changes under alkaline conditions (Handayani et al., 2024). Saponins were confirmed by the formation of stable foam resulting from their ability to reduce surface tension (Budiyanto et al., 2025). Triterpenoids were further verified by the appearance of a brown ring in the Salkowski test, indicating a sulfonation reaction (Handayani et al., 2024).

According to Muawanah et al. (2023), the mixing of plant extracts may alter phytochemical profiles due to differences in relative concentrations, solubility, and interactions among secondary metabolites within the mixture. The newly formed phytochemical profile suggests that combining the two extracts not only integrates the bioactive compounds present in each plant but may also increase the diversity of detectable metabolites. The simultaneous presence of alkaloids, flavonoids, saponins, tannins, and triterpenoids in the combined extract may promote synergistic interactions among these compounds, thereby enhancing their biological activities compared to individual extracts. Such interactions can influence the stability and bioactivity of secondary metabolites, which may contribute to the improved pharmacological potential of the combined extract, including its antibacterial activity (Endesei et al., 2024).

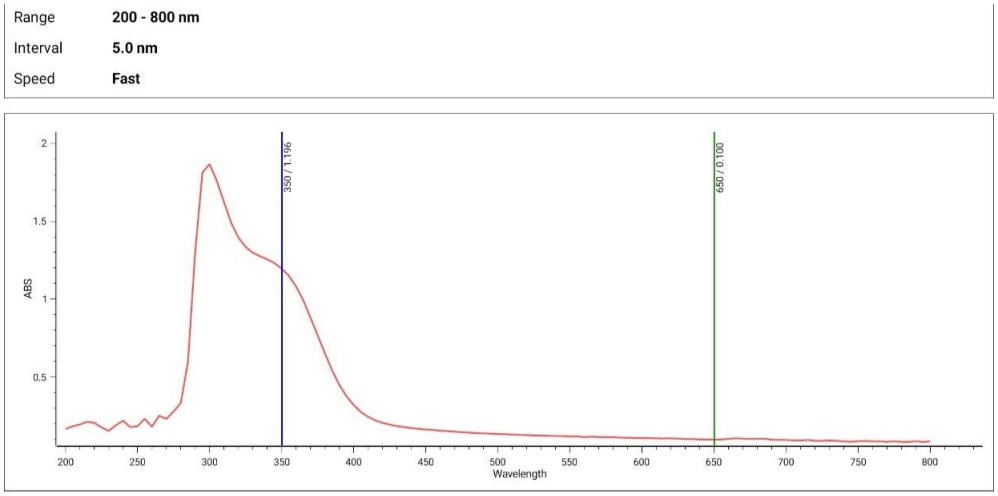
UV-Vis Spectrophotometry-2



Sample	ABS(350)	ABS(650)
Sente p3 1	0.178	0.007

Figure 2. UV-Vis Spectrophotometry Graph of Phytochemical Extract from Sente Leaves

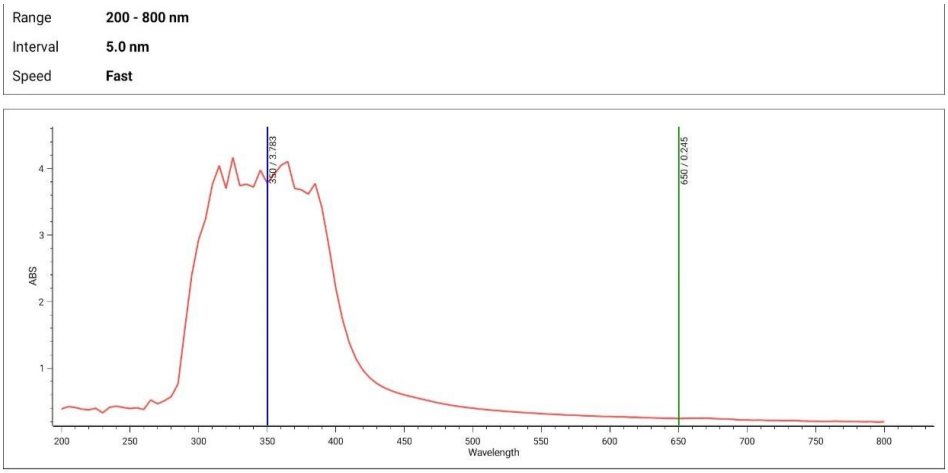
In Figure 1, the absorption curve of the *sente* leaf extract shows a low peak of 0.178 at 350 nm and a very low peak of 0.007 at 650 nm. These results indicate low phytochemical concentrations, likely dominated by non-aromatic compounds or dilute extract. This interpretation aligns with UV-Vis analyses of Indonesian herbal plant extracts, such as *Mentha spicata*, where weak UV absorption suggests limited flavonoid content without sharp peaks. The overall flat curve indicates a lack of extensive electron-π conjugation, differing from the richer phytochemical profiles observed in extracts such as those from grapes (Wardani *et al*., 2025).



Sample	ABS(350)	ABS(650)
Jambu biji p2 1	1.196	0.100

Figure 3. UV-Vis Spectrophotometry Graph of Phytochemical Extract from Guava Leaves.

Meanwhile, Figure 2 shows the absorption curve of guava leaf extract with a high peak of approximately 1.196 at 350 nm and a low peak of 0.100 at 650 nm, indicating the presence of strong flavonoids and phenolic compounds. Flavonoids typically absorb in the UV range of 300–400 nm due to π–π* transitions in their aromatic rings. The peak at 350 nm aligns with other studies on guava leaf fractions using UV-Vis to identify flavonoids such as quercetin, supporting its antioxidant and antimicrobial potential. The low absorption at 650 nm suggests minimal presence of visible light-absorbing compounds like anthocyanins or chlorophyll (Raghupathy *et al*., 2024).



Sample	ABS(350)	ABS(650)
Kombinasi p1 1	3.783	0.245

Figure 4. UV-Vis Spectrophotometry Graph of Combined Phytochemical Extract from Guava and Sente Leaves.

Finally, Figure 3 shows the spectrum of the combined *sente* leaf and guava leaf extract, yielding the highest peak of 1.722 at 350 nm and 0.031 at 650 nm, indicating a dominant additive effect with increased absorption

compared to individual extracts. This reflects phytochemical synergy, similar to findings in mixed plum leaf extracts where UV-Vis confirmed elevated total flavonoid content post-combination. The sharper peak at 350 nm suggests phenolic compound stability in the mixture, holding potential for further pharmacological applications (Furi *et al*., 2018).

FTIR Test Result-3

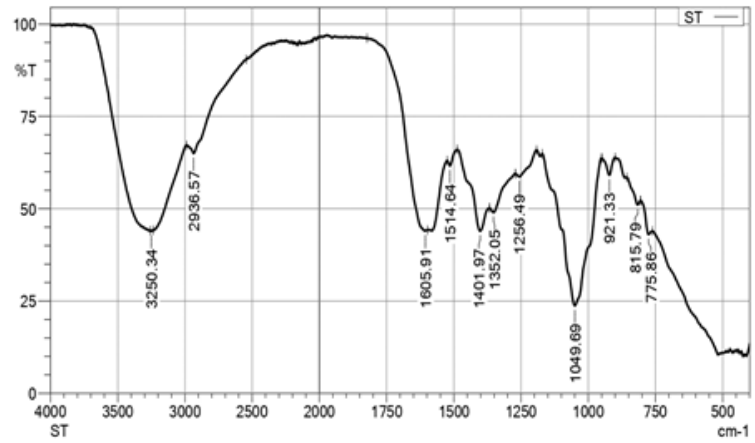


Figure 5. FTIR Results of the *sente* leaf Extract.

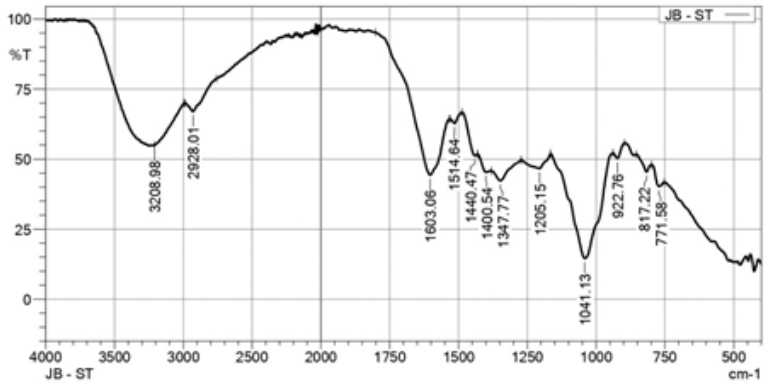


Figure 6. FTIR Results of the Guava leaf Extract.

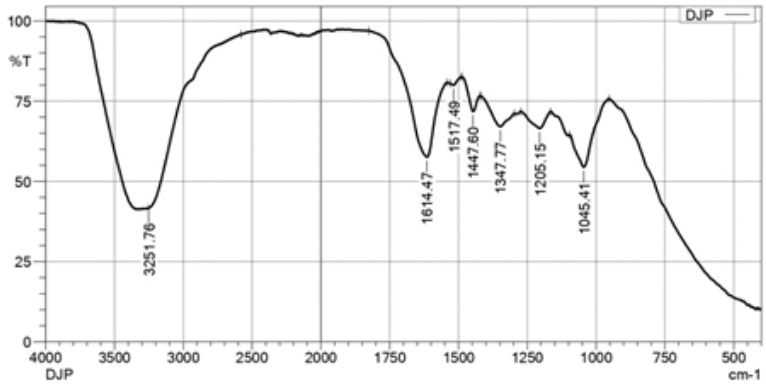


Figure 7. FTIR Results of the combined Sente leaf and guava leaf Extract.

FTIR analysis of *sente* leaf extract, guava leaf extract, and their combination revealed relatively similar functional group patterns with differences in absorption intensity. All three spectra displayed broad absorption bands in the 3200–3400 cm^{-1} region, indicating the presence of –OH groups characteristic of phenolic

compounds, flavonoids, and tannins, which are consistent with phytochemical screening and UV-Vis analysis results.

Absorption bands in the 2920–2850 cm^{-1} region indicate aliphatic C–H vibrations associated with triterpenoids and saponins, which were more prominent in figure 2 guava leaf extract and figure 3 the combined extract. Absorptions in the 1600–1650 cm^{-1} region indicate aromatic C=C and/or C=O bonds related to flavonoid and tannin aromatic ring structures. Additionally, bands in the 1000–1200 cm^{-1} region correspond to C–O vibrations commonly found in alcohols, phenols, and glycosidic compounds.

Overall, the FTIR spectra of all three samples confirm the presence of major functional groups constituting secondary metabolite compounds, with stronger absorption intensities observed in guava leaf extract and the combined extract, indicating higher phenolic compound content. These findings support the phytochemical screening data and reinforce the potential of sente leaf extract, guava leaf extract, and their combination as sources of natural bioactive compounds.

CONCLUSIONS

Leaf extracts of *sente* were identified to contain flavonoids, tannins, and triterpenoids, while guava leaves exhibited a more comprehensive profile of secondary metabolites, including alkaloids, flavonoids, saponins, tannins, and triterpenoids. The combination of both extracts indicated synergistic active compounds, supported by UV-Vis absorption peaks at 321–369 nm characteristic of conjugated flavonoid and polyphenol systems. Overall, both plants demonstrate substantial potential as natural herbal medicine raw materials due to their significant bioactive content.

Authors' Contributions: Adelia Permata Dewi, Majida Ramadhan, Qonita Mukrimatul Hijjah, and Naura Dinada Zulfa Salsabila designed the study. Adelia Permata Dewi, Qonita Mukrimatul Hijjah, and Naura Dinada Zulfa Salsabila carried out the laboratory work and wrote the manuscript. Majida Ramadhan, Faisal, and Nafisa supervised the study and reviewed the manuscript. All authors read and approved the final version of the manuscript.

***Competing Interests*:** The authors declare that there are competing interests.

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