Biomedical Sciences and Clinical Medicine 2026;65(1):51-57.
doi: 10.12982/BSCM.2026.05
https://he01.tci-thaijo.org/index.php/CMMJ-MedCMJ/index

BSCM
Biomedical Sciences and Clinical Medicine

Original Article

Open Access

Comparative Study of Guava Leaf Bioactive Extracts Using Ultrasonication Assisted Extraction

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Received: October 2, 2024;
Revised: August 9, 2025;
Accepted: August 18, 2025

ABSTRACT

OBJECTIVE Guava (*Psidium guajava* L.), a member of the Myrtaceae family, is grown worldwide and is widely consumed in India because of its good nutritional value as well as its medicinal properties. various parts of the guava tree, e.g., its root, bark, leaves, and fruit, have been found to be rich in pharmacological properties. Despite the health benefits, parts of the tree other than the guava fruit are not widely used by people. Guava leaves are generally considered as a non-conventional food with high nutritive value, medicinal properties, essential oils and good amounts of bioactive compounds.

METHODS In this study, these bioactive compounds were extracted by ultrasound-assisted extraction (UAE) using three different solvents (water, ethanol and methanol) and the extracts were tested for finding the bioactive content in it.

RESULTS This study found that dilute methanol (50 : 50) extracts contain significant amounts of flavonoids, alkaloids, saponins and terpenoids. Ethanol extracts showed good quantities of tannins and aqueous extract showed good extraction of total phenolic content.

CONCLUSIONS These findings show that guava leaves are rich in bioactives compounds which can be extracted using environmentally friendly methods which can be used in food and pharmaceutical industries

KEYWORDS guava leaves, bioactives, extraction, ultrasonication assisted extraction, flavonoids

INTRODUCTION

Guava (*Psidium guajava* L.) is a well-known fruit belonging to the Myrtaceae family and is grown in tropical and subtropical regions of many countries. India holds first position worldwide in the cultivation of guava. Uttar Pradesh, Madhya Pradesh, Maharashtra and Bihar are some of the important guava growing states in the country. Allahabad District of Uttar Pradesh is famous for growing one of the best quality guava fruit in the world (1). Many pharmacological properties have been reported for the bark, roots, leaves and fruit of the plant (2, 3). Generally, guava leaves are considered as a non-conventional food as many people consume guava fruit, ignoring the nutritive as well as the medicinal properties of guava leaves. Guava leaves play an important role in treating diarrhea, gastroenteritis and other digestive problems (4, 5). They also exhibit antioxidant, anti-inflammatory and antimicrobial actions (6).

Guava leaves are rich in nutrients as they contain moisture, ash, fat, protein and carbohydrates (7). They also contain good concentrations of Ca, P, Mg, Fe, vitamin C and vitamin B-complex. They

are considered a good source of bioactive compounds such as alkaloids, flavonoids, terpenoids and phenolic compounds (8). They also contain significant amounts of essential oils (9, 10). Many functional properties, e.g., neuroprotective (11), antioxidant (12), decreasing blood pressure (13), antimicrobial (14), anti-carcinogenic, and anti-inflammatory (15) have been shown in bioactive compounds present in various parts of the plant including leaves, stems, fruits, and flowers which can be extracted using different methods.

Both conventional and non-conventional techniques are known for extracting the bioactive compounds. Methods used in conventional techniques include soxhlet extraction, maceration and hydrodistillation among others. However, extraction with these techniques generally takes long time, is costly, requires large amounts of solvent and is inefficient (16). To overcome these limitations, non-conventional extraction methods have been introduced, including ultrasound, microwave, enzyme, and pulsed electric field assisted extraction among others. These methods require less extraction time, use less solvent, have high extraction rates and reduce thermosensitive compound degradation. These methods are known as "Green Extraction" as they function in accordance with standards set by the U.S. Environmental Protection Agency (17).

The ultrasonication assisted extraction (UAE) technique is easy to use and inexpensive, reduces the quantity of solvent needed, shortens the extraction time, produces a high extraction yield, improves extract quality, allows selective extraction, uses low temperatures thus avoiding heat damage and reducing bioactive compound loss (18). It is an environmentally friendly method and hence is considered a biorefining technology. In this process, ultrasound waves at frequencies higher than 20 kHz are used to disrupt the cell wall of the plant, increasing the solvent's capacity to permeate the cells, resulting in a higher extraction yield. This process can be easily used in a laboratory using an ultrasonic bath which is easy to operate (19). In this study, the UAE approach was used for extracting bioactive compounds from guava leaves.

METHODS

Collection of plant material

Many varieties of guava are found in India. The variety "*Allahabad Safeda*" was chosen for this study. The leaves of this variety were collected from the Krishi Vigyan Kendra Farm Science Center in Banasthali Vidyapith, Newai, Rajasthan, India. The guava leaves were handpicked from the tree and were first washed under running water to remove all the dirt and soil, then were gently rinsed with distilled water. The leaves were subsequently dried in a hot air circulation oven at 80°C for 15-16 hours. The dried leaves were then crushed and ground in an electric grinder to obtain a coarse powder which was then used to extract the bioactive compounds. In this study, all results are expressed as dry weight.

Extraction of bioactive compounds

The bioactive components were extracted from the guava leaves by following and modifying methods given by Mehmood et al. (20). In this study, three extracts were prepared using distilled water, ethanol and diluted methanol: aqueous extract, ethanol extract and diluted methanol extract, respectively.

Aqueous extract (AE): 50g of dried sample was dissolved in 100ml of distilled water and the solution was heated in a water-bath at 100°C for 2 hours.

Ethanol extract (EE): 50g of dried sample was dissolved in 100ml of ethanol and was allowed to stand for 48 hours at room temperature (25-30°C).

Diluted methanol extract (DME): 50g of dried sample was dissolved in 100ml of diluted methanol (1:1 v/v) and the solution was allowed to stand for 48 hours at room temperature (25-30°C).

After the above steps, each extract was exposed to an ultrasonic water bath for 2 hours following which the solvent was evaporated using a rotary evaporator at 69 rpm with a bath temperature of 45°C until the extract was concentrated. After that, each extract was dried in a hot air circulation oven at 45-50°C to remove excess moisture. Extracts were then stored in airtight containers for further use.

Estimation of quantities of bioactive compounds

Total quantities of phenolic compounds were evaluated using the Folin-Ciocalteu method. Flavonoid content was determined using the aluminum chloride method. The quantities of alkaloids, saponins and tannins were determined following procedures described in Singh et al. (21).

To measure the terpenoid content, 100 mg of dried sample was dissolved in 9 mL of ethanol for 24 hours. The mixture was then filtered using Whatman filter paper. The resulting filtrate was extracted with 10 mL of petroleum ether using a separating funnel. The ether extract was isolated and completely dried in pre-weighed glass vials. After the ether was evaporated, the total terpenoid content (mg/g) was determined using the formula: (22).

Statistical analysis

All experiments were performed in triplicate, and the results are expressed as mean ± standard deviation (SD). The data were analyzed using Microsoft Excel. Descriptive statistics were used to calculate the mean and SD and to assess the variability among replicates.

RESULTS

Fresh guava leaves of the Allahabad Safeda variety were collected, dried and converted into a fine powder as shown in Figure 1. The powder was then used in making aqueous, ethanol and diluted

methanol extracts to determine the bioactive compounds present. Table 1 provides a comparative analysis of the bioactive compounds in AE, EE, and DME of guava leaves.

The yields obtained for aqueous, ethanol and diluted methanol extracts were 14%, 17% and 22%, respectively.

Total phenolic content. The total phenolic content of guava leaves (Figure 2) shows that the aqueous extract contains the highest quantity of phenolic compounds with 186 ± 0.4 mg GAE/g,

$$\text{Formula: Terpenoids content (mg/g)} = \frac{\text{Weight of terpenoids residue (mg)}}{\text{Weight of sample (g)}} \times 1{,}000$$

Figure 1. Fresh (left) and dry (right) guava leaves

Table 1. Bioactive compound analysis of AE, EE, and DME of guava leaves

Bioactive compound	AE	EE	DME
Total phenolic content (mg GAE/g)	186±0.4	135±0.5	164±0.7
Flavonoids (mg/g)	6.84±0.5	5.87±0.7	11.39±0.2
Alkaloids (mg/g)	11.40±0.2	10.86±0.1	11.89±0.8
Saponins (%)	0.58±0.6	0.61±0.5	1.12±0.2
Tannins (mg/L)	276±0.1	312±0.3	290±0.4
Terpenoids (mg/g)	3.81±0.3	3.88±0.6	3.96±0.7

Values are presented as means ± SD
AE, aqueous extract; EE, ethanol extract; DME, diluted methanol extract

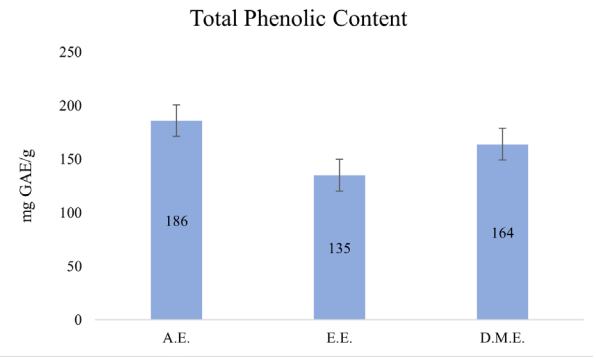

Figure 2. Total phenolic content in aqueous, ethanol and diluted methanol extract of guava leaves

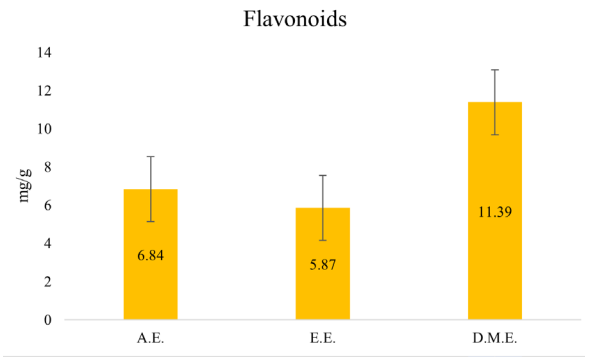

Figure 3. Flavonoid content in aqueous, ethanol and diluted methanol extract of guava leaves

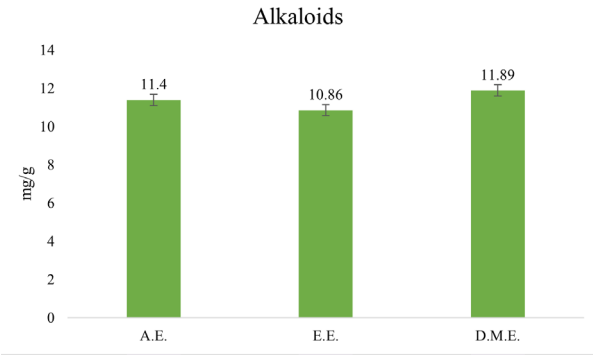

Figure 4. Alkaloid content in aqueous, ethanol and diluted methanol extract of guava leaves

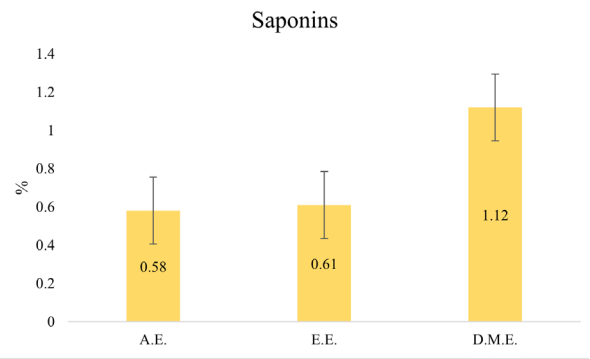

Figure 5. Saponin content in aqueous, ethanol and diluted methanol extract of guava leaves

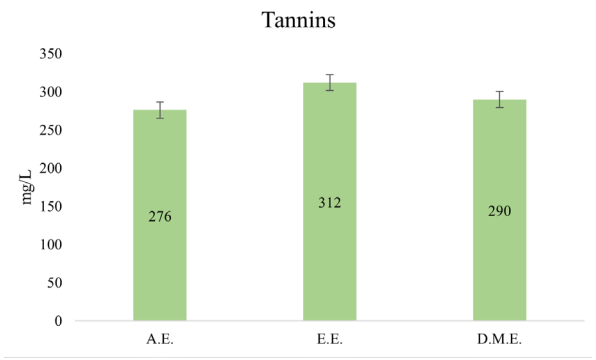

Figure 6. Tannin content in aqueous, ethanol and diluted methanol extract of guava leaves

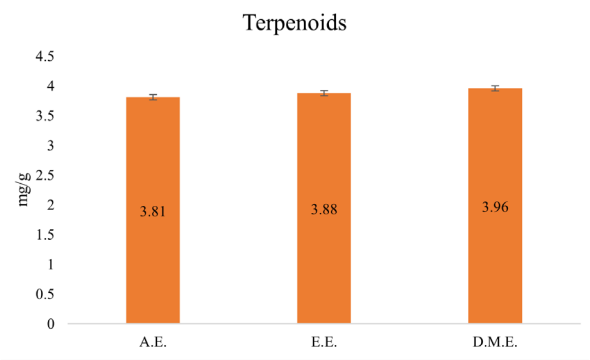

Figure 7. Terpenoid content in aqueous, ethanol and diluted methanol extract of guava leaves

followed by diluted methanol extract with 164 ± 0.7 mg GAE/g and ethanol extract with 135 ± 0.5 mg GAE/g.

Flavonoids. The flavonoid content present in guava leaves is shown in Figure 3. Diluted methanol extract had the highest content of flavonoids with 11.39 ± 0.2 mg/g followed by aqueous extract with 6.84 ± 0.5 mg/g. Ethanol extract had the lowest flavonoid content, 5.87 ± 0.7 mg/g.

Alkaloids. The alkaloid content present in guava leaves is shown in Figure 4. The highest content of alkaloids was present in diluted methanol extract with 11.89 ± 0.8 mg/g, followed by the aqueous extract with 11.4 ± 0.2 mg/g and ethanol extract which had the lowest alkaloids content, 10.86 ± 0.1 mg/g.

Saponins. The saponin content present in guava leaves is shown in Figure 5. The highest presence of saponins was in diluted methanol extract with 1.12 ± 0.2%, followed by ethanol extract with 0.61 ± 0.5%, and aqueous extract which had the lowest saponin content with 0.58 ± 0.6%.

Tannins. The tannin content of guava leaves is shown in Figure 6. The highest content of tannins

was present in ethanol extract, 312 ± 0.3 mg/L, followed by diluted methanol extract with 290 ± 0.4 mg/L and aqueous extract, 276 ± 0.1 mg/L, the lowest tannin content.

Terpenoids. The terpenoid content of guava leaves is shown in Figure 7, a maximum terpenoid content of 3.96 ± 0.7 mg/g in diluted methanol extract followed by 3.88 ± 0.6 mg/g in ethanol extract and the minimum terpenoids content, with 3.81 ± 0.3 mg/g, in aqueous extract.

DISCUSSION

The findings of total phenolic content results coincide with a similar study done using hydroethanolic extract conducted by Amaral et al. (23) where total phenolic content was found to be 185 and 150 mg GAE/g in 50% and 70% hydroethanolic extract, respectively. Another study conducted by Hartati et al. (24) reported total phenolic compound of guava leaves ethanolic extract to be 146.7 mg GAE/g. Slightly higher concentrations of total phenolic content were observed by Cerio et al., 2016 where a significantly higher concentration of total phenolic content, 304 mg GAE/g, was found in etOH/H_2O 80:20 (v/v) solvent. The findings of flavonoids in this study are in alliance with the findings by Díaz-de-Cerio et al. (25) where the flavonoids content in n-hexane leaf extract of crystal guava variety was observed to be 9.68 mg/g. Slightly higher flavonoid content, 18.66 mg/g, was observed by Pandhi et al. (26) using the UAE method. Not many studies were found related to other bioactive compounds of guava leaves.

CONCLUSIONS

The study successfully demonstrated the efficiency of UAE in isolating bioactive compounds from guava leaves using different solvents. The results indicate that diluted methanol is the most effective solvent for extracting flavonoids and alkaloids, while ethanol is superior for tannin extraction, and aqueous best depicts high total phenolic content. This finding highlights the importance of solvent selection in maximizing the yield of specific bioactive compounds. The high content of these compounds in guava leaves suggests their significant potential for use in health-promoting applications. Future research should focus on exploring the therapeutic benefits of these extracts and optimizing extraction methods for industrial applications. The findings promote the utilization of guava leaves, which are often discarded, thus adding value to guava cultivation and contributing to sustainable agricultural practices. This study had some limitations: it was carried out on a small scale under laboratory conditions which may not fully reflect real-world applications. Also, only basic methods were used to analyze the total amounts of bioactive compounds.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the staff of Krishi Vigyan Kendra of Banasthali Vidyapith, Newai, Rajasthan, India, for their support in identification of the guava variety. We would also like to give special thanks to Mr. Vimal Jain for providing technological knowledge during our experiment.

FUNDING

No fundings provided.

CONFLICTS OF INTEREST

No conflicts of interest are associated with the authors for this investigation.

AUTHOR CONTRIBUTION

S.K.: collected the raw material, performed the experiment, evaluated the result and wrote the manuscript; P.S.: helped in drafting the manuscript and guided in the experiment. E.S.C.: supervised the work, guided in the experiment and revised the final manuscript.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article.

INSTITUTIONAL REVIEW BOARD STATEMENT

Ethical review and approval were waived for this study, as it did not involve human participants or animals.

INFORMED CONSENT STATEMENT

Not applicable.

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