Green Extraction of Bioactive Compounds from Guava Fruit and Leaves: A Comparative Study Using Ultrasound-Assisted Extraction

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ABSTRACT

Introduction: Guava (*Psidium guajava L.*) is a tropical plant widely recognized for its nutritional and medicinal value. Its fruit and leaves are rich sources of bioactive compounds, including phenols, flavonoids, alkaloids, tannins, and terpenoids, which exhibit antioxidant, anti-inflammatory, and antimicrobial properties. Despite the fruit's widespread use, guava leaves remain underutilized due to limited awareness of their health benefits

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Aim: This study focused on extracting bioactive compounds from guava fruit and leaves using the ~~Ultrasound-Assisted Extraction (UAE) technique by using aqueous (water) as solvent and compares~~Ultrasound-Assisted Extraction (UAE) technique using aqueous (water) as a solvent and comparing the bioactive compounds present in guava fruit and leaves.

Methodology: This study ~~uses fresh guava fruit and leaves which were dried and grinded separately into a powder. Then the bioactives compounds were extracted using the UAE technique and water was used as solvent and the extract of guava fruit and leaves were prepared that were~~used fresh guava fruit and leaves that were dried and ground separately into a powder. Then, the bioactive compounds were extracted using the UAE technique, and water was used as a solvent. The extract of guava fruit and leaves was prepared, which was further used for analysis.

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Results: The results revealed significant yields, with guava leaves showing higher phenolic content ~~with 192 mg GAE/g, alkaloid content with 11.89 mg/g, tannin content with 276 mg/l and terpenoid content with 3.81 mg/g, while guava fruit had elevated flavonoid content with 7.9 mg/g and saponin content with 0.71%~~(192 mg GAE/g), alkaloid content (11.89 mg/g), tannin content (276 mg/l), and terpenoid content (3.81 mg/g), while guava fruit had elevated flavonoid content (7.9 mg/g) and saponin content (0.71%).

Conclusion: The findings ~~shows~~ show that the guava leaves aqueous extract ~~contain~~ contains a high content of bioactive ~~compound~~ compounds as compared to the guava fruit aqueous extract. Future studies can explore the therapeutic applications of guava bioactive compounds in pharmaceuticals and functional foods. Additionally, optimizing green extraction methods like UAE for industrial-scale applications can enhance sustainable utilization.

Keywords: *Aqueous extract, Bioactive compounds, Nutritional, Medicinal, Phenols*

1. INTRODUCTION

Guava (*Psidium guajava* L.) belonging to Myrtaceae family is generally grown worldwide in ~~the~~ tropical regions ~~an~~ and India is considered ~~as~~ the highest source of cultivating guava. Moreover, the Allahabad district of Uttar Pradesh is famous of growing the best quality of guava. The whole guava tree is considered to have many medicinal properties ~~like different its~~ leaves, roots, bark, fruit and stem are famous for treating diarrhea, diabetes, ~~ulcer~~ulcers, ~~stomachache~~ stomachaches etc. (Khanna S *et al.,* 2022). The shape of guava fruit is round or oval according to the species and they are 4-12 cm long with bitter to sweet taste and on the other hand, guava leaves are oval, elliptical and dark green ~~in color~~. Guava fruit and leaves are considered to be highly nutritious and rich in the treatment of various diseases (Arshiya S; 2013).

Guava fruit is rich in minerals and vitamins like calcium, iron, phosphorus, ascorbic acid, β carotene, niacin, lycopene, vitamin A, and fiber. Along with minerals and vitamins, they are very good ~~source~~ sources of bioactive compounds like phenols, flavonoids, alkaloids, saponins, tannins, terpenoids, quercetin, linolenic acid, cectin, linoleic acid etc. They are very famous and frequently used for their medicinal properties like antioxidant, anti-inflammatory and antimicrobial actions (Kumar M *et al*., 2021). It is very helpful in treating digestive problems. Guava leaves generally come under non-conventional food as they are not consumed by many people due to the unawareness of its nutritional and health benefits. Guava leaves are rich in nutrients as they contain moisture, ash, fat, protein and carbohydrates (Shabbier H *et al*., 2020). They also contain good ~~concentration~~ concentrations of Ca, P, Mg, Fe, vitamin C and vitamin B-complex. They are considered as good source of bioactive compounds like alkaloids, flavonoids, terpenoids and phenolic compounds (Morais ME *et al*., 2017). They also contain good amount of essential oils (Naseer S *et al*., 2018). Many functional properties like neuroprotective (Chen FY *et al*., 2021), antioxidant (Zhang X *et al*., 2021), decreasing blood pressure (Aekthammarat D *et al*., 2020), antimicrobial (Pham DQ *et al*., 2021), anti-carcinogenic, anti-inflammatory (Hamzalıoglu A and Gokmen V, 2016) are shown by bioactive compounds present in various parts of plants like leaves, stem, fruits, flowers and can be extracted by using different methods.

Bioactive compounds are the secondary metabolites of plant system that helps in improving survival capacity and they can be extracted either by the ~~convention~~ conventional or non-conventional methods (Rodriguez Garcia SL and Raghavan V; 2022). Conventional extraction techniques such as Soxhlet extraction, maceration, and hydro-distillation are time-consuming, require large amounts of costly solvents, and often yield inefficient results (Agregán R et al., 2021). To address these limitations, non-conventional extraction methods have been developed, including ultrasound-assisted, microwave-assisted, enzyme-assisted, and pulsed electric field-assisted extraction. These innovative techniques significantly reduce extraction time and solvent usage, enhance extraction efficiency, and minimize the degradation of thermosensitive compounds (Fu X et al., 2021). Collectively referred to as “Green Extraction,” these methods

align with the Environmental Protection Agency (EPA) standards in the USA, promoting environmentally sustainable practices.

Ultrasound-Assisted Extraction (UAE) is an easy-to-use and cost-effective technique that reduces solvent consumption and extraction time, delivers high yields, enhances extraction quality, and enables selective extraction. Operating at low temperatures, it minimizes heat damage and loss of bioactive compounds (Carriara CA et al., 2021). As an environmentally friendly method, UAE is recognized as a bio-refining technology. This process employs ultrasound waves with frequencies above 20 kHz, which disrupt plant cell walls, enhancing the solvent's ability to penetrate cells and improving extraction efficiency. UAE is straightforward to perform in a laboratory setting using an ultrasonic bath (Hadidi et al., 2020). In this research, the UAE technique was utilized to extract bioactive compounds from guava fruit and leaves effectively.

2. METHODS AND MATERIALS

2.1 Collection and preparation of plant material

The "Allahabad Safeda" variety of guava fruit and its leaves were collected from the Krishi Vigyan Kendra of Banasthali Vidyapith, Newai, Rajasthan, India. The guava fruit and its leaves were handpicked from tree and were washed separately by running water to remove the impurities like dirt and soil. They were then separately rinsed with distilled water and air-dried at 80℃ for 20 and 15 hours respectively. After the completion of drying, both were ~~grinded~~ ground into a fine powder and stored separately for ~~the~~ further use.

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2.2 Extraction of bioactive compound

In this study, the aqueous extract of both guava fruit and leaves were prepared to extract the bioactive compounds and they were extracted by Ultrasonication-Assisted Extraction (UAE) technique. To prepare the extract, dried guava fruit and leaves powders were dissolved in 100 ml of distilled water separately and then these ~~solutions~~ mixture ~~were~~ was heated in water-bath at 100℃ for 2 hours. Then both extracts ~~was~~ were exposed to an ultrasonic water bath for 2 hours and then the solvent was evaporated by using a rotary evaporator at 69rpm with bath temperature at 45℃ till it was concentrated. After this, each extract was dried in an ~~air circulated~~air-circulated oven at 45-50℃ for the removal of excess moisture and they were stored in airtight containers for further use.

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2.3 Estimation of bioactive compounds

Total phenolic compounds were evaluated using the Folin-Ciocalteu method, ~~Flavonoids~~ flavonoid content was determined using aluminium chloride method, ~~Alkaloids~~alkaloids, ~~Saponins~~saponins, and ~~Tannins~~ tannins were determined by following the procedures explained in Singh., *et al*.

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To determine the terpenoid content, 100 mg of dried sample was dissolved in 9 ml of ethanol and left to stand for 24 hours. The mixture was subsequently filtered through Whatman filter paper. The filtrate was then subjected to extraction with 10 ml of petroleum ether using a ~~separating~~

separatory funnel. The ether layer was collected and dried completely in pre-weighed glass vials (final weight: wf). After the evaporation of the ether, the total terpenoid yield (%) was calculated using the formula: (Wi-wf/wi x 100) (Malik et al., 2017).

3. RESULTS AND DISCUSSION

The process of making the aqueous extract of guava fruit and leaves are shown in figure1 and 2 respectively.

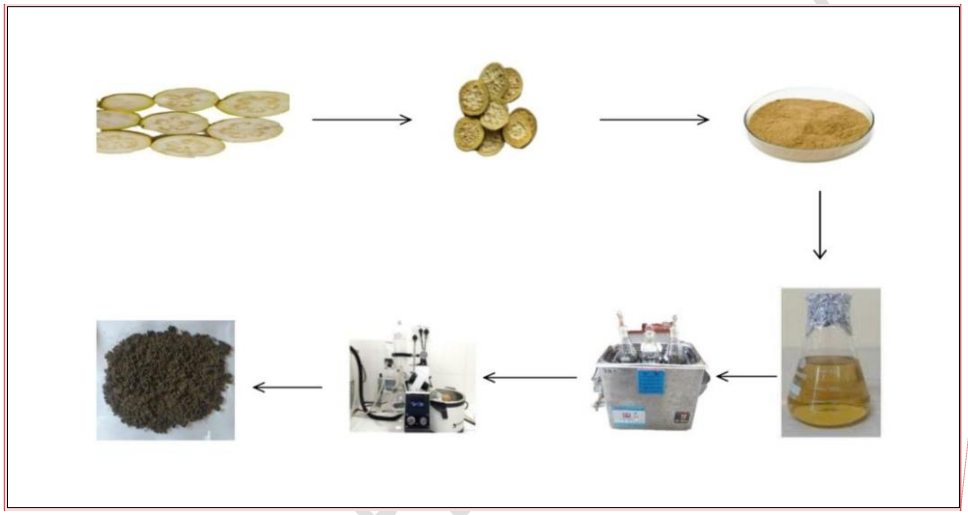

Figure 1: Processing of guava fruit aqueous extract

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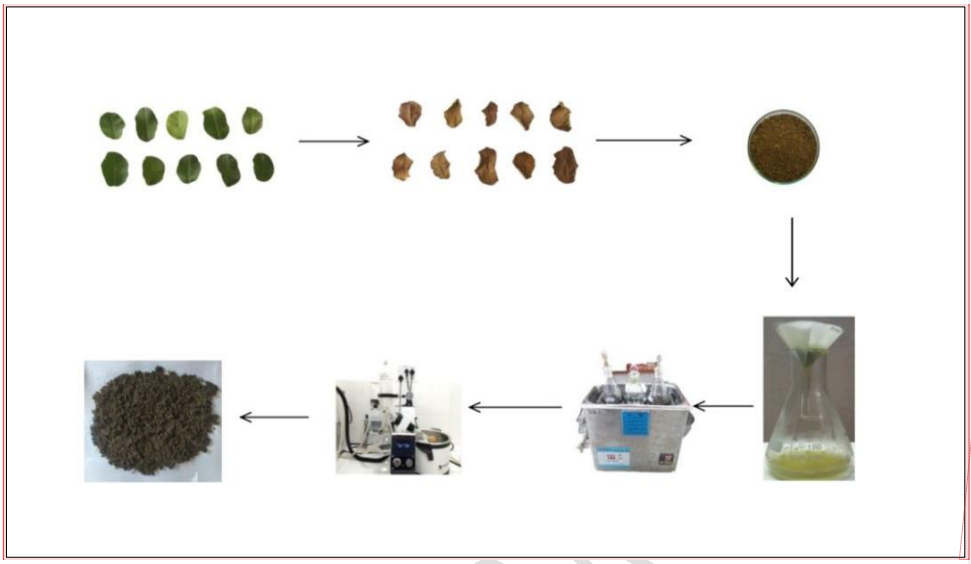

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Figure 2: Processing of guava leaves aqueous extract

3.1 Flavonoids: The flavonoid content of guava fruit and leaves aqueous extract is depicted in figure 3, which shows the presence of high ~~flavonoids~~ flavonoid content in guava fruit aqueous extract with 7.90 mg/g followed by the guava leaves aqueous extract with 6.89 mg/g. Sornapudi DS and Srivastava M; 2022 conducted a study to estimate phytochemicals in different leaf extracts and the ~~flavonoids~~ flavonoid content of guava aqueous extract came to be 54.35 mg RE/100g.

3.2 Alkaloids: The ~~alkaloids~~ alkaloid content of guava fruit and leaves aqueous extract were evaluated and shown in figure 3, which states that the guava leaves aqueous extract ~~contain~~ contains high ~~alkaloids~~ alkaloid content with 11.89 mg/g as compared to the guava fruit aqueous extract with 9.26 mg/g.

3.3 Saponins: The saponin content present in guava fruit and leaves aqueous extract is shown in figure 3, and its value are represented in (%). The value shows the presence of saponins content to be high in guava fruit aqueous extract with 0.71% as compared to the guava leaves aqueous extract with 0.58%.

3.4 Tannins: The presence of tannins content in guava fruit and leaves aqueous extract is shown in figure 3, and the values ~~shows~~ show the high content in guava leaves aqueous extract with 276 mg/l as compared to the guava fruit aqueous extract with 271 mg/l.

3.5 Terpenoids: The terpenoids content present in guava fruit and leaves aqueous extract are showed in figure 3, which shows the presence of high terpenoids content in guava leaves aqueous extract with 3.81 mg/g followed by the guava fruit aqueous extract with 3.71 mg/g.

3.6 Total Phenolic Content: The total phenolic content present in guava fruit and leaves aqueous extract is shown in figure 3, and the value is determined in ~~the~~ terms of gallic acid as it was taken as the standard. The values of the high presence of total phenolic content in guava leaves aqueous extract with 192 mg GAE/g as compared to the guava fruit aqueous extract with 186 mg GAE/g. Sornapudi DS and Srivastava M; 2022 conducted a study ~~which~~ that shows phytochemicals analysis in different leaf extracts and the ~~phenlic~~ phenolic content of guava aqueous extract was 23.55 mg GAE/100g while Ademiluyi A O *et al*., (2016) conducted the study to compare the antihypertensive and antioxidant properties of phenolic extracts of guava fruit and leaves of different varieties in which the lead and fruit extracts were used for analyses and the results showed total phenolic content with the values of 53.4 mg GAE/100g content of small white guava.

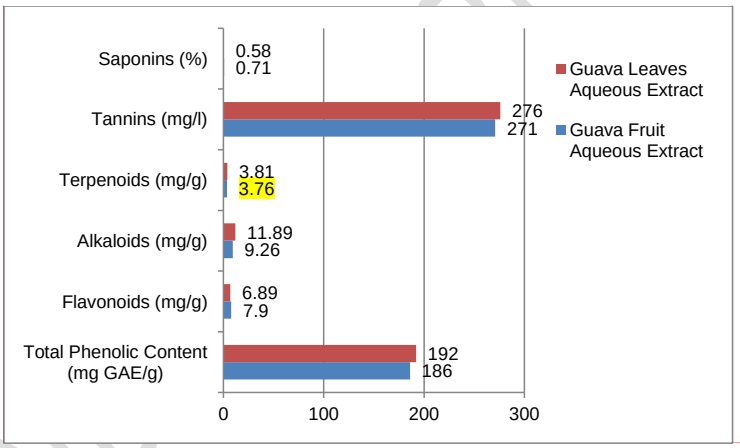

Figure 3: Comparative analysis of bioactive compounds in aqueous extracts of guava fruit and leaves

4. CONCLUSION

Guava (*Psidium guajava L.*) is a versatile plant recognized for its nutritional and medicinal properties, with various parts of the plant, including its fruit and leaves, widely used in traditional medicine, also they are rich in bioactive compounds such as phenols, flavonoids, alkaloids, saponins, and terpenoids, guava exhibits significant antioxidant, antimicrobial, and anti-inflammatory properties. This study employed Ultrasound-Assisted Extraction (UAE), an eco-friendly and efficient method, to extract bioactive compounds from guava fruit and leaves. The

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findings demonstrated that guava leaves contain higher levels of phenolic and alkaloid compounds, while the fruit exhibited greater flavonoid and saponin content. These results highlight the potential of guava as a valuable source of bioactive compounds with applications in pharmaceuticals, nutraceuticals, and functional foods. The study validates the effectiveness of UAE as a green extraction technology and emphasizes the importance of sustainable methods for maximizing the utilization of natural resources.

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