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Efficacy evaluation of extraction technologies for guava (*Psidium guajava* L.) leaves extract

Shikha Pandhi, Arvind Kumar[♦] and Dinesh Chandra Rai

Department of Dairy Science and Food Technology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi-221005, Uttar Pradesh, India

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Abstract

Guava leaves contain significant levels of antioxidant phenolic compounds and flavonoids. Given its strong antioxidant activity, isolating phytochemicals from guava leaves for functional food development would be extremely advantageous. The goal of this study was to assess how different extraction procedures influenced the phytochemical composition and antioxidant activity of *Psidium guajava* L. leaves. The study revealed that the highest extraction yield (21.33%), TPC (17.81 mg GAE/ml), and TFC (23.41 mg of QE/ml) was observed in UMAE extract. Similar results were obtained for antioxidant activity with the highest DPPH (84.03%), SOSA (80.78%), and ABTS (97.69%) inhibition. The preliminary screening of phytochemicals and FTIR analysis had indicated the existence of phenols, flavonoids, alcohols, aromatic compounds, etc. These phytochemicals possess antioxidant properties and can serve as an essential component for the development of functional and nutraceutical products.

1. Introduction

The use of plants for medical and therapeutic purposes to cure illnesses and enhance human health is known as a phytomedicine. Plants produce phytochemicals as a defense against microbial infestations, and they have therapeutic potential in medicinal and nutraceutical preparations (Shakya, 2016). Human illnesses like atherosclerosis, arthritis, diabetes, cancer and other age-related disorders can be reduced by eating foods that contain phytochemicals with possible antioxidant effects. The guava tree (*Psidium guajava* L.), which belongs to the Myrtaceae family, is a highly distinctive plant that is used for its therapeutic and nutritional properties. Guava is a tropical fruit. The roots, bark, leaves, stem, and fruits of the guava have been used to cure stomach aches, diabetes, diarrhoea, etc. Leaves accumulate the most bioactive substances, known as secondary metabolites, of all plant parts (Kumar *et al.*, 2021). Guava leaves contains high concentration of polyphenols and flavonoids associated with potent antioxidant activity. The main active substances in guava leaves are phenols such as gallic acid, flavonoids as quercetin (Raj *et al.*, 2020), caffeic acid, guaijaverin, tannins, carotenoids, and triterpenoids (Kumar *et al.*, 2021). Because of their biological actions, polyphenols and flavonoids have emerged in recent years being one of the most promising components for the functional food industry (Pimpley and Murthy, 2021). Isolating phytochemicals from guava leaves for functional food development would be very beneficial, considering its high antioxidant activity.

Extraction is an essential step for isolating bioactives from plant matrix. The goal of an extraction procedure is to acquire the greatest antioxidant activity and maximal concentration of target components (Musa *et al.*, 2011). At present, the most basic classification is between classical extraction processes and non-traditional “greener” extraction procedures (Rocchetti *et al.*, 2019). The conventional methods like maceration, decoction and Soxhlet are most common but are associated with some drawbacks whereas, unconventional extraction techniques like pressurized liquid extraction, supercritical fluid extraction, microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE) offer advantages of mechanization, improved selectivity, better efficiency, and less solvent consumption (Alara *et al.*, 2021). The purpose of this study was to evaluate and compare the extraction yields, phytochemical profile, antioxidant potential and total phenolic and flavonoid content of guava leaves extracts obtained using different extraction technologies, such as maceration, stirring, heat and stirring, Soxhlet extraction, boiling method, homogenizer (HAE), ultrasound-assisted (UAE), microwave-assisted (MAE), and ultrasound and microwave (UMAE) coupled extraction method. Despite the consistent usage of guava in daily life, a full biochemical characterization of its leaf extracts as a source of phytochemicals, as well as the impact of extraction procedures on its phytochemical content, has yet to be further explored, published, and compared.

2. Materials and Methods

2.1 Materials and Chemicals

Fresh guava leaves were collected from the agriculture farm of the Institute of Agricultural Sciences of Banaras Hindu University, Varanasi. The leaves were dried for 12 h at 40°C. The dried leaves were crushed into powder, which was then passed through a standard screen of 20 mesh size and stored in an airtight container until the

Corresponding author: Dr. Arvind Kumar

Assistant Professor, Department of Dairy Science and Food Technology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi-221005, Uttar Pradesh, India

E-mail: arvind1@bhu.ac.in

Tel.: +91-9793583702

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Email: ukaaz@yahoo.com; Website: www.ukaazpublications.com

research was completed. This investigation employed analytical-grade chemicals and reagents.

2.2 Extraction methods of guava leaves

Nine different extraction treatments were employed with slight modifications as described below.

2.2.1 Maceration

Ten grams of triturated leaf powder and 100 ml of solvent (water) were taken. The mixture was kept in the dark at room temperature for 24 h, with sporadic shaking with a glass agitator (Sharma and Cannoo, 2016).

2.2.2 Stirring-assisted extraction

Ten grams of powder mixed with 100 ml of solvent (water) was subjected to continuous shaking at room temperature in a shaking incubator for 24 h at 120 rpm (Chuah *et al.*, 2020).

2.2.3 Heat and stirring-assisted extraction

Ten grams of leaf powder were added to 100 ml of water and stirred for 30 min at 250 rpm and 60°C on a magnetic stirrer.

2.2.4 Soxhlet extraction

With minor modifications, Alara *et al.* (2018) explained the methodology used. In a Soxhlet extractor at 60°C for 4 h, the same amount of solute and solvent combination (10 g solute in 100 ml water) were continuously extracted.

2.2.5 Homogenizer-assisted extraction (HAE)

The method employed was adopted from Eyiz *et al.* (2020) with slight modifications. The solute and solvent mixture (10 g of powder in 100 ml water) was subjected to homogenization in an ultraturrax (IKA T25 digital, Staufenim Breisgau, Germany).

2.2.6 Boiling method

In this extraction method, 10 g of powdered sample was boiled in a boiling waterbath with 100 ml water for 20 min (Seifipour *et al.*, 2020).

2.2.7 Microwave-assisted extraction (MAE)

About 10 g of sample was added to 100 ml of solvent (water) and soaked for 20 min. The resulting mixture was microwave irradiated at 110°C for 1 min at 3 different intervals (Pandhi and Poonia, 2019).

2.2.8 Ultrasound-assisted extraction (UAE)

The extraction was done using a probe ultrasonicator. Ten grams of leaf powder was added to 100 ml of water as solvent in a glass beaker. The probe was immersed in the resulting mixture for 30 min at 70% sonication amplitude (Machado *et al.*, 2019).

2.2.9 Ultrasound and microwave-assisted extraction (UMAE)

The same procedure was followed as in ultrasound-assisted extraction except additional treatment was given by exposing the ultrasonicated mixture to microwave irradiation for 10 seconds (Dong *et al.*, 2021).

All of the extracts from each technique were centrifuged for 15 min at 6,000 rpm and filtered through Whatman paper no. 1. The resulting extract was stored at 4°C until further use.

2.3 Phytochemical screening-Qualitative

The qualitative phytochemistry assays were carried out according to the procedures described (Modi *et al.*, 2018; Shaikh and Patil, 2020) to confirm the occurrence of phenols, flavonoids, alkaloids, glycosides, tannins, saponins, carbohydrates, quinones, and coumarins (Table 1).

2.4 Extraction yield of extract

The extraction yield (%) was calculated for each extract as per the method given by Patle *et al.* (2020).

2.5 Total phenolic content (TPC) of extract

The TPC was estimated using the method discussed by Hinneburg *et al.* (2006) with slight modifications. The TPC was expressed in mg of gallic acid equivalent (GAE)/ml of extract.

2.6 Total flavonoid content (TFC) of the extract

The TFC was evaluated using the method described by Kamtekar *et al.* (2014). The TFC of extract was expressed as mg of quercetin equivalents (QE)/ml of extract.

2.7 Antioxidant activity of the extract

Three different methods were employed to evaluate the antioxidant value of extracts obtained using different extraction methods. The antioxidant activity of the sample extract was determined by using DPPH free radical scavenging (Shirazi *et al.*, 2014), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical scavenging (Vikas *et al.*, 2017), and Superoxide anion scavenging activity (SOSA)(Adeosun *et al.*, 2016) method expressed as % inhibition as described in the method.

2.8 Fourier transform infrared spectroscopy (FTIR)

One of the most effective ways for identifying functional groups in substances is the Fourier transform infrared spectrophotometer. For FTIR analysis, the dried aqueous extract was employed. To make a clear sample disc, 10 mg of dried extract powder was encapsulated in 100 mg of KBr pellet. The powdered sample from each plant specimen was put in an FTIR spectroscope (Shimadzu, IR Affinity1, Japan) with a scan range of 400 to 4000 cm⁻¹ and a resolution of 4 cm⁻¹ (Shareef and Bhavya, 2021).

2.9 Statistical analysis

Triplicate readings for each technique were taken. The findings were examined statistically in IBM SPSS Statistics 22 using one-way ANOVA and Duncan's post hoc test to investigate for significant differences at a $p < 0.05$ level.

3. Results

3.1 Phytochemical screening

Initial qualitative screening to determine the presence of phytochemicals can be used to assess the plants' medicinal potential. This study screened bioactive components that provide plants physiologically active properties, and the results were obtained. The phytochemicals screened in the guava leaves extract using different extraction methods was indicated in Table 1. The result obtained reveals the presence of phenols, tannins, flavonoids, carbohydrates, tannins, quinones, coumarins, and the absence of alkaloids and phytosterols. From the qualitative screening of the phytochemicals presence of different phytochemicals can be found, but relevant information regarding the effect of the extraction method can not be observed. Hence, the extracts were subjected to quantitative analysis.

Table 1: Comparative screening of phytochemicals in guava leaves extract extracted using different extraction methods

Phytocompound	Extraction methods								
	Maceration	Stirring-assisted	Heat and stirring-assisted	Boiling	Soxhlet	HAE	MAE	UAE	UMAE
Saponins	+	+	+	+	+	+	+	+	+
Alkaloids	–	–	–	–	–	–	–	–	–
Flavonoids	+	+	+	+	+	+	+	+	+
Phenols	+	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+	+
Carbohydrates	+	+	+	+	+	+	+	+	+
Quinones	+	+	+	+	+	+	+	+	+
Phytosterols	–	–	–	–	–	–	–	–	–
Coumarins	+	+	+	+	+	+	+	+	+

Table 2: Effect of different extraction method on yield, TPC and TFC of guava leaves extract

Extraction method	Yield (%)	TPC (mg gallic acid equivalent /ml of extract)	TFC (mg of quercetin equivalent /ml of extract)
Maceration	11.28 ± 0.90 ^e	6.59 ± 0.15 ^e	4.72 ± 0.36 ^h
Stirring-assisted	14.17 ± 0.86 ^d	8.12 ± 0.51 ^d	6.53 ± 0.36 ^g
Heat and stirring-assisted	15.71 ± 0.67 ^{bc}	9.22 ± 0.48 ^d	8.23 ± 0.39 ^f
Homogenizer-assisted (HAE)	15.21 ± 0.27 ^{cd}	8.13 ± 0.68 ^d	6.73 ± 0.05 ^g
Boiling	16.49 ± 0.27 ^{bc}	12.63 ± 1.22 ^c	11.56 ± 0.42 ^e
Soxhlet	15.74 ± 0.58 ^{bc}	14.15 ± 0.42 ^b	16.32 ± 1.16 ^d
Microwave-assisted	15.42 ± 0.96 ^{cd}	14.70 ± 0.55 ^b	20.85 ± 0.69 ^b
Ultrasound-assisted	16.98 ± 0.6 ^b	15.35 ± 1.26 ^b	18.66 ± 1.62 ^c
Ultrasound and microwave-assisted (UMAE)	21.33 ± 1.15 ^a	17.81 ± 0.70 ^a	23.41 ± 1.31 ^a

All values are expressed as Mean ± SD (n=3). Different superscripts in the same column indicate significant difference ($p < 0.05$).

Table 3: Effect of different extraction method on antioxidant activity of guava leaves extract

Extraction method	DPPH inhibition (%)	ABTS inhibition (%)	SOSA (%)
Maceration	71.96 ± 0.37 ^f	90.54 ± 0.49 ^d	69.94 ± 0.63 ^e
Stirring-assisted	74.09 ± 1.52 ^e	91.36 ± 0.65 ^d	70.82 ± 0.56 ^{de}
Heat and stirring assisted	75.60 ± 1.19 ^d	91.85 ± 0.94 ^d	71.14 ± 0.46 ^d
Homogenizer-assisted (HAE)	78.18 ± 0.70 ^c	91.62 ± 2.29 ^d	75.76 ± 0.48 ^c
Boiling	78.57 ± 0.65 ^{bc}	97.03 ± 0.88 ^{ab}	76.00 ± 0.72 ^c
Soxhlet	77.53 ± 0.78 ^c	94.70 ± 0.97 ^c	77.14 ± 0.27 ^b
Microwave-assisted (MAE)	78.58 ± 0.55 ^{bc}	95.37 ± 0.80 ^{bc}	76.99 ± 0.43 ^b
Ultrasound-assisted (UAE)	80.08 ± 0.54 ^b	96.98 ± 0.36 ^{ab}	77.93 ± 0.44 ^b
Ultrasound and microwave-assisted (UMAE)	84.03 ± 0.76 ^a	97.69 ± 0.48 ^a	80.78 ± 0.62 ^a

All values are expressed as Mean ± SD (n=3). Different superscripts in the same column indicate significant difference ($p < 0.05$).

3.2 Extraction yield

The extraction yield of *P. guajava* prepared by different extraction methods is summarized in Table 2. UMAE method gave the highest extraction yield (21.33 ± 1.15%) compared to other methods due to enhanced penetration of solvent into the solute matrix and better solubilization of compounds into the solvent with a significant difference from other methods. The lowest extraction yield was

observed in maceration (11.28 ± 0.90%) and stirring (14.17 ± 0.86%) due to the lack of heat employed during extraction (Chuah *et al.*, 2020).

3.3 Total phenolic and total flavonoid content of extract

The total phenolic content and total flavonoid content were tabulated in Table 2. The significantly highest TPC was observed in UMAE extract (17.81 ± 0.70 mg GAE/ml), followed by the UAE (15.35 ±

1.26 mg GAE/ml), MAE (14.70 ± 0.55 mg GAE/ml) and Soxhlet (14.15 ± 0.42 mg GAE/ml) with insignificant differences. There was no significant difference between the TPC of extract obtained using these methods. The lowest TPC values were obtained in the maceration method and stirring-assisted extraction. The reason for this could be the lower penetration of the solvent into the solute matrix, resulting in lower yield as well as lower phenols in the extract. In the case of TFC, similar results were reported with significantly higher values for UMAE (23.41 ± 1.31 mg QE/ml). The reason for this could be the coupled effect of both green technologies (ultrasound and microwave) that provides better penetration of solvent into the solid matrix. The lowest TFC values were obtained in maceration, stirring, heat and stirring-assisted, and HAE.

3.4 Antioxidant activity

The antioxidant activity was assessed by three *in vitro* methods such as DPPH, ABTS, and SOSA per cent inhibition. The values obtained for different antioxidant assays for extracts obtained using different extraction methods were depicted in Table 3. The highest antioxidant activity in terms of DPPH, SOSA, and ABTS % inhibition was observed in UMAE extract with values of 84.03 ± 0.76 , 80.78 ± 0.62 , and $97.69 \pm 0.48\%$, respectively. The lowest values were

observed in maceration and stirring assisted as both of these techniques are conventional and lack the use of heat or any other mode of external force compared to other methods that either make use of heat or high-shear forces as homogenizers, sound, and microwaves.

3.5 FTIR analysis of the extract

The FTIR spectrum observed gives the “fingerprint” of the functional groups and chemical compounds present in the guava leaves extract obtained using UMAE. The X-H stretching area, the triple-bond region, the double-bond region, and the fingerprint region are generally separated into four sections in the mid-infrared spectrum in the range $4000\text{--}400\text{ cm}^{-1}$ (Pandhi and Poonia, 2019). The FTIR spectrum of the UMAE guava leaves extract was shown in Figure 1 and the peaks identified as illustrated in Table 4. The fundamental vibrations at wavelengths 2924.79 and 2852.05 cm^{-1} indicate the presence of alkane with C-H stretching. The possible compounds showing C-H stretching in this region indicate phenols, flavonoids, CH_3 and CH_2 (Nagpal *et al.*, 2021). The O-H bending band obtained at 1358.77 cm^{-1} indicate the presence of phenol. The C-H bending at 1731.52 cm^{-1} confirms the occurrence of aromatic compounds. The other compounds that were inferred from the obtained FTIR spectrum were alkyne, primary and secondary alcohols, anhydride, *etc.*

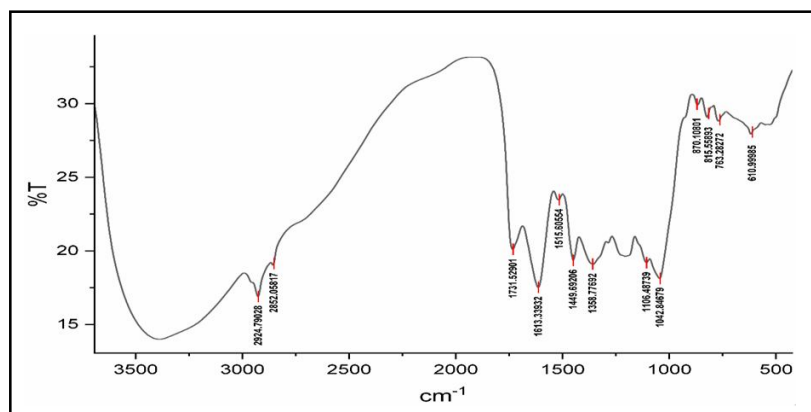


Figure 1: FTIR graph of UMAE guava leaves extract.

Table 4: Functional groups identified in UMAE guava leave extract using FTIR

Wave number (cm^{-1})	Wave number range (cm^{-1})	Functional group	Compound class
610.99	690-515	C-Br stretching	Halo compound
763.28	750 ± 20	=C-H bending	Monosubstituted
815.55	840-790	C=C bending	Alkene
870.10	880 ± 20	C-H bending	Tri or di-substituted
1042.84	1050-1040	CO-O-CO stretching	Anhydride
1106.48	1085-1050	C-O stretching	secondary alcohol
1358.77	1390-1310	O-H bending	phenol
1449.69	1450	C-H bending, stretching	CH_3 , CH_2 , flavonoids, aromatic ring
1515.60	1550-1500	N-O stretching	Nitro compound
1613.33	1650-1600	C=C stretching	Conjugated alkene
1731.52	2000-1650	C-H bending	Aromatic compound
2852.05	3000-2840	C-H stretching	Alkane (Flavonoids, polyphenols, CH_3 , CH_2)
2924.79	3000-2840	C-H stretching	Alkane (Flavonoids, polyphenols, CH_3 , CH_2)

4. Discussion

Flavonoids and other plant phenolic compounds have strong antioxidant capabilities and can trap free radicals and reactive oxygen species (Kiran *et al.*, 2019). One of the most essential aspects in obtaining high-quality natural antioxidants is the extraction method. Simple, quick, and ecologically friendly approaches should be adopted. The selected extraction procedure, however, should have a high ability to remove the most active chemicals without destroying them. Shortening the extraction time, reducing solvent usage, increasing extraction yield, and improving the quality of the extracts are all advantages of the perfect approach (Nantitanon *et al.*, 2010). The study reveals that *P. guajava* leaves serve as a potent source of antioxidant phytochemicals. In this study, different extraction methodologies have been employed to evaluate their effect on the phytochemical and antioxidant profile. The UMAE method has shown to be the best amongst all the extraction methods given its high efficiency for the extraction of plant bioactives. UMAE method used was based on green extraction techniques that provide better efficiency and yields due to the occurrence of a phenomenon known as “cavitation” in which high temperature, high shear forces, and free radicals work to disrupt the cell wall giving a high extraction yield coupled with electromagnetic microwaves for uniform heating (Russo *et al.*, 2019). The coupling of these two methods has offered better efficiency in terms of higher extraction yield, TPC and TFC, and antioxidant activity. The presence of a high amount of phenols and flavonoids has been linked with enhanced antioxidant activity. A study conducted by Pandhi and Poonia (2019) reported that antioxidant activities of the plant extracts were positively related with total phenolics and flavonoid contents as higher phenolic content generally relates to high antioxidant activity. The FTIR spectrum obtained indicated the presence of phenols, flavonoids, alcohols, aromatic compounds, alkane, *etc.* A study conducted by Nantitanon *et al.* (2010) supports the finding that ultrasonication process offers better extraction efficiency compared to conventional maceration and Soxhlet method. Consequently, UMAE was recommended as the most effective extraction procedure. The knowledge gathered from this work is predicted to be useful for extracting natural antioxidants from guava leaves on a micro scale as well as on a commercial scale.

5. Conclusion

The findings show that *P. guajava* is a rich source of plant bioactives that might be employed in the development of functional foods and nutraceuticals. Phenols, tannins, flavonoids, coumarins, quinones, saponins, and other compounds were found in the preliminary phytochemical screening. The antioxidant activity of UMAE extract was highest, relating to its high flavonoids and phenolic content. The presence of phenols, flavonoids, alcohols, alkene, halo and nitrocompounds, aromatic compounds, alkane, and other substances was confirmed from the FTIR spectrum. To summarize, the UMAE approach was shown to be the most effective of all the extraction strategies for isolating bioactive chemicals with high antioxidant activity from guava leaves for commercial use.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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