A comparative analysis of the phytochemical composition and *in -vitro* antioxidant activity of various parts of *Psidium guajava* Linn found in Sri Lanka

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Received on 09 May 2024; received in revised form 24 September 2024, accepted 07 October 2024.

Abstract
The incidence of disease is growing worldwide, particularly in developing countries. *Psidium guajava* L. (Myrtaceae) usually recognized as guava, is a good snack for patients due to its low-calorie and high soluble fiber contents. The current study was to assess the antioxidant activity with the phytochemical characteristics of methanolic, ethanolic, and aqueous extracts of various parts of *Psidium guajava*. After shade drying, each part was ground into a powder and extracted separately with various solvents using cold extraction method. Standard screening methods were followed and the colorimetric method was used to access the total contents of phytochemicals. The findings demonstrated that tannins, glycosides, phenols, and flavonoids were existing in all of the extracts from the various components. The total tannin, phenolic, flavonoid, and alkaloid contents of *Psidium guajava*'s leaves and bark were higher than those of the plant's fruits and seeds. Furthermore, when compared to other parts, methanolic leaf extract had the highest DPPH and ABTS antioxidant activity since it had the lowest IC50 values (19.17±0.258 and 356.16±0.156 mg/ml, respectively). Finally, this work offers a thorough examination of the antioxidant and phytochemical characteristics of the Sri Lankan-grown *Psidium guajava* variety, which the scientific community can utilize for further research.

Keywords: Antioxidant, Different parts, Phytochemical, *Psidium guajava*, Sri Lanka.

Introduction

Since ancient times, people have utilized medicinal herbs also known as therapeutic plants in conventional medical procedures. Plants produce a wide range of chemical substances for several uses, including as defense against diseases, fungi, insects, and herbivorous mammals (Rajkumar et al., 2023). Several phytochemicals have been discovered that may or may not have biological function (Rajkumar et al., 2021). Because a single plant has a wide range of phytochemicals, the effects of using the entire plant as medicine are still unknown (Kumar et al., 2023). Medicinal plants are rich in bioactive phytochemicals, often known as bio nutrients (Sanmugarajah and Rajkumar, 2022; Rajkumar et al., 2022 and 2022 b). The last two to three decades' worth of research has shown how important these phytochemicals are for preventing chronic diseases (Rajkumar et al., 2021). Dietary fiber, antioxidants, neuropharmacological agents, anticancer, detoxifying, and immunity-potentiating mediators are the main groups of phytochemicals having disease prevention properties. These functional agents are divided into several different chemical classes, each with a variety of potencies. Some of

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these phytochemicals have multiple uses such as cosmetics, health and hygiene, fragrance, and food supplements (Camilleri and Blundell, 2024). Antioxidants are becoming more and more well-liked all over the world due to their potential function in the food business and human health. Antioxidants are constituents that can stop or slow the oxidation of easily oxidized compounds, even in very small amounts (Francenia Santos-Sánchez, 2019). The human body has been shown to produce free radicals and extra reactive oxygen species through various physiological and biochemical processes (Phaniendra et al., 2015).

Psidium guajava L. The plant, usually denoted to as guava (Myrtaceae) (Kumar et al., 2021). A well-known traditional medication utilized in a number of indigenous medical systems is *Psidium guajava* L. Its leaves and bark have both been utilized medicinally for a very long time and are still used now (Daswani et al., 2017). It is originally from Central America, but it is now extensively produced, spread, and the fruits in the tropics of the world enhance the meals of millions of people. The traditional uses of *Psidium guajava* L., commonly denoted to as the "poor man's apple" of the tropics, have a long history and a significant number of them have been supported by scientific research (Shruthi et al., 2013). Guava cultivars and variants, i.e., commonly consumed, wild, and introduced varieties, are plentiful in Sri Lanka. Many cultivars are available in common-guava, *Psidium guajava* L. (Shanthirasekaram et al., 2021). Guava cultivars and variants abound in Sri Lanka. However, there is little scientific evidence on guava leaves, bark, fruits and seeds.

The ultimate objective of the current investigation was to assess the phytochemical properties and antioxidant activity of methanolic, ethanolic, and aqueous extracts of different *Psidium guajava* parts that are used in disease management. This comparative analysis will aid in the selection of plant parts with the highest medicinal potential, possibly guiding the development of novel therapeutic applications and pharmaceutical formulations. Furthermore, it may provide treasured evidence for biodiversity protection and sustainable consumption of this medicinally significant herb.

Figure 1. Selected parts (Seeds, leaves, fruits and bark) of the *Psidium guajava* L. plant

Materials and Methods

The various parts of the *Psidium guajava* L. plant, including as its leaves, bark, fruits, and seeds, were collected from the Jaffna District in December 2021, during the maturity season. Three replicates of herbarium were prepared. Their identification was confirmed botanically by the Department of Botany, Faculty of Science, University of Jaffna, Sri Lanka.

Preparation of different parts of plant materials

After being cleansed with tap water, freshly harvested leaves, seeds, fruits, and bark were allowed to air dry for a week at room temperature. They were then dried for five days in a 40°C Oven (Memmert). The dried plant sources were pulverized and sieved through up to 80 meshes using an electric grinder. It was stored separately in waterproof containers for further examination after being ground into a fine powder.

Preparing aqueous, methanolic, and ethanolic extracts

A different conical flask had twenty grams of the ground plant material, to which 100 milliliters of methanol, ethanol, and water were added. To aid in

shaking, the flask was covered and placed in the shaker for two hours every five days. Whatman filter paper was used to filter the extracts after five days.

Qualitative and quantitative phytochemical analysis of selected parts Psidium guajava L.

A preliminary phytochemical screening of the ethanol, methanol, and water extracts of the leaves, bark, seeds, and fruits of *Psidium guajava* L. powders was done using standard laboratory procedures (Table 1) to determine the presence of the various phytochemicals. (Harborne, 1998; Raaman, 2006; Kamal, 2014; Tiwari et al., 2011; Savithramma et al., 2011; Obouayeba et al., 2015; Kumar et al.,2013; Singh & Kumar, 2017; Silva et al, 2017; Gul et al., 2017; Maria et al., 2018 and Kumar et al., 2018).

Table 1. Tests for Phytochemical Screening

Phytochemicals	Tests
Flavonoids	Alkaline reagent test
Phenols	Ferric chloride test
Tannins	Ferric chloride test
Alkaloids	Mayer's test
Terpenoids	Chloroform test
Anthraquinones	Ammonium hydroxide test
Saponin	Foam test/Frothing test
Quinones	Alcoholic KOH test
Coumarins	NaOH test
Glycosides	Keller and Kiliani test
Steroids	Salkaowski test
Anthocyanin	HCl test
Reducing sugar	Fehling test
Xanthoprotein	Xanthoproteic test
Carboxylic acid	Sodium bicarbonate test - Effervescence test
Protein and amino acids	Xanthoproteic test

The total concentration of phytochemicals was evaluated using standard laboratory protocols from the ethanol, methanol, and water extracts of the leaves, bark, seeds, and fruits of *Psidium guajava* powders. Table 2 presents the results of the analysis.

Estimation of antioxidant action of selected parts of Psidium guajava L.

DPPH Free Radical Scavenging Assay

Using a spectrophotometer to detect absorbance at 517 nm, the radical scavenging activity of *Psidium guajava* L. plants towards 1,1-diphenyl-2-picrylhydrazyl (DPPH) was used to assess the plants' level of antioxidant activity.

ABTS scavenging activity

The technique outlined by Re et al. (1999) was used to measure the ABTS scavenging activity at 734 nm.

Median inhibitory concentration (IC50) which is sample concentration mandatory to scavenge 50% of the free radicals was determined from the graph for DPPH and ABTS activity.

Statistical Analysis

Data for three replicates of each *Psidium guajava* L. plant parts were statistically analyzed using one-way analysis of variance (ANOVA) by using mini tab 17 software and Turkery's multiple comparisons at probability value (pd•0.05). The outcomes were stated as Mean ± Standard deviation (SD).

Result and Discussion

Qualitative and quantitative analysis of phytochemical constituents of selected parts of Psidium guajava L.

The chemical composition of guava extracts is evaluated through a qualitative phytochemical screening, as mentioned in Table 3. The findings demonstrated that while tannins, glycosides, phenols, and flavonoids were existing in all of the extracts from the various components, anthocyanins

Table 2. Tests for Phytochemical Estimation

Phytochemical Contents	Test	References
Phenols	Folin–Ciocalteu colorimetric method	(Singleton et al., 1999)
Flavonoids	Aluminum colorimetric method	(Badarinath et al., 2010)
Tannin	Folin- Ciocalteu colorimetric method	(Kavitha & Indira, 2016)
Akaloids	Spectrophotometric method	(Edeoga et al., 2005 and Aliyu et al.,2008)

Table 3. Result of the phytochemical screening of the *Psidium guajava* L.

S.N.	Phytochemical	Ethanolic extract				Methanolic extract				Aqueous extract			
		L	S	B	F	L	S	B	F	L	S	B	F
1.	Alkaloid	++	+	++	-	++	+	++	+	+	-	+	-
2.	Glycoside	+	+	+	+	++	+	+	+	+	+	+	+
3.	Phenol	++	+	++	++	+++	+	+	+	++	+	+	++
4.	Flavonoid	++	+	++	+	++	+	++	+	++	+	++	+
5.	Tannins	++	+	+++	+	++	+	+++	+	++	+	+++	+
6.	Terpenoid	-	+	-	+	-	+	-	+	-	+	-	+
7.	Anthraquinone	+	-	+	-	+	-	+	-	+	-	+	+
8.	Saponin	++	-	+	+++	+	-	++	+	++	-	+	+
9.	Quinone	+	-	+	-	++	-	++	-	+	-	+++	+
10.	Steroids	-	+	-	+	-	+	-	+	-	-	-	+
11.	Anthocyanin	-	-	-	-	-	-	-	-	-	-	-	+
12.	Reducing Sugar	-	+	-	+	-	+	-	+	-	+	-	+
13.	Xanthoprotein	+	-	+	-	+	-	+	-	+	-	+	-
14.	Carboxylic Acid	-	-	-	-	-	-	-	-	-	-	-	-
15.	Coumarin	-	+	-	+	-	+	-	+	-	+	-	+

L- Leave; S – Seed; B – Bark; F – Fruit; (+++) abundant; (+) present; (-) absent

and carboxylic acid were not present. And also, it was observed that methanolic extraction yielded more phytochemicals than both aqueous and ethanolic extracts. Aqueous and alcoholic seed extract revealed the fewest constituents, while methanolic leaf extract revealed the greatest number of phytoconstituents. The results for the presence of secondary metabolites were almost identical for the methanolic and ethanolic extracts, while the aqueous extract showed less because the color intensity was lower. This could be because some chemicals aren't fully soluble in aqueous solvent.

Table 3 describes the qualitative phytochemical screening used to assess the chemical composition of guava extracts. The results exposed the occurrence of active compounds in the three diverse extracts. In Table 3, the findings demonstrated that while tannins, glycosides, phenols, and flavonoids were present in all of the extracts from the various components, anthocyanins and carboxylic acid were not present. In addition, high tannin concentrations were observed in the ethanolic, methanolic, and aqueous bark extracts. Aqueous and alcoholic seed extract revealed the fewest constituents, while methanolic leaf extract revealed the greatest number of phytoconstituents. The results for the presence of secondary metabolites were almost identical for the methanolic and ethanolic extracts, while the aqueous extract showed less because the color intensity was lower. This could be because some chemicals aren't fully soluble in aqueous solvent.

Table 4. Quantitative estimation of phytochemicals of *Psidium guajava L.*

Parts	Extracts	Phenolics (µg/ml)	Tannins (µg/ml)	Flavonoids (µg/ml)	Alkaloids (mg/g)
Bark	Ethanolic	56.190 ± 0.881	1150.70 ± 0.871	19.780 ± 0.776	296.8 ± 0.543
	Methanolic	53.797 ± 0.545	1060.80 ± 0.757	22.970 ± 0.619	
	Aqueous	61.467 ± 0.961	953.04 ± 0.797	24.887 ± 0.465	
Leaf	Ethanolic	125.520 ± 0.622	1006.80 ± 0.551	32.880 ± 0.478	128.0 ± 0.335
	Methanolic	131.330 ± 0.577	899.33 ± 0.577	33.200 ± 0.794	
	Aqueous	118.670 ± 0.577	909.93 ± 0.681	29.480 ± 0.934	
Fruit	Ethanolic	78.033 ± 0.569	327.72 ± 0.737	1.305 ± 0.006	63.20 ± 0.241
	Methanolic	17.727 ± 0.818	310.75 ± 0.626	1.363 ± 0.032	
	Aqueous	35.833 ± 0.814	470.44 ± 0.991	1.363 ± 0.032	
Seed	Ethanolic	7.077 ± 0.172	131.54 ± 0.528	0.437 ± 0.015	26.40 ± 0.126
	Methanolic	8.093 ± 0.314	167.16 ± 0.620	1.033 ± 0.008	
	Aqueous	12.607 ± 0.761	170.77 ± 0.551	2.133 ± 0.131	

Mean ± Standard Deviation; Means are significantly different ($P < 0.05$)

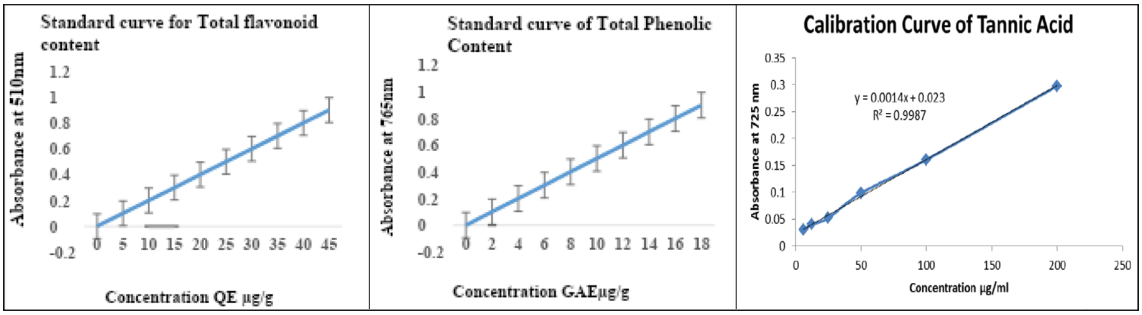

Figure 2 Standard curve for total phenol, flavonoid and Tannin (Nantongo et al., 2018; Pratyusha, 2022). It has been demonstrated that alterations in growth circumstances, particularly in the availability of nitrogen (N), impact the content of phenolic composites in plant tissues (Larbat, 2012).

Table 4 shows that the bark's methanolic, ethanolic, and aqueous extracts had the highest tannin content, whereas the leaves had the highest quantities of flavonoids, phenolic compounds, and tannins. Additionally, the bark has the maximum concentration of tannins and alkaloids. However, the least quantity of tannins, phenols, flavonoids, and alkaloids is found in seeds. These findings indicate that the total tannin, phenolic, flavonoid, and alkaloid contents of *Psidium guajava* are higher in the leaves and bark than in the fruits and seeds of the plant.

The maximum flavonoid content was shown (35.200±0.794 µg/ml) in methanolic leaves extracts. Flavonoids have antioxidant properties; they may lessen oxidative stress in cells. Methanolic extracts had the highest extraction yields due to the highest levels of flavonoids and phenolics that were found in them. This may be explained by the compounds' greater solubility in methanol as compared to the other tested solvents. When combined, these results point to methanol as the ideal solvent for removing bioactive substances from *Psidium guajava* L. plant parts. Low concentrations of hydrogen peroxide are found naturally in the human body, the air, water, plants, microbes, diet, and beverages (Gov. UK. 2009). The methanolic extract of guava leaves was noticeable to have a higher H_2O_2 scavenging activity (Venkatachalam et al., 2012).

The result clearly indicated that the highest amount of tannin in ethanolic (1150.7±0.871µg/ml); methanolic (1060.80±0.757 µg/ml) and aqueous extracts (953.04±0.797 µg/ml) of bark than other parts. The study's findings showed that *Psidium guajava* L. bark extract has the highest concentration of tannins. Proteins and other organic substances, such as amino acids and alkaloids, are bound to and precipitated by tannin, a bitter polyphenolic chemical originate in plants (Adamczyk et al., 2017). This combination of tannin and protein has long-lasting antioxidant properties (Okuda & Ito, 2011**;** Amarowicz and Janiak, 2019; Tong et al., 2022). The current investigation provided a systematic record of the tannin's relative capacity to scavenge free radicals in *Psidium guajava* L. bark extract and in addition, could quicken the healing of wounds and burns which may be due to the occurrence of tannins (Xiaowen Su et al., 2017). Further, trees that accumulate tannins in their bark are resistant to bacterial and fungal infections (Raitanen et al., 2020). In this instance, the tannins prevent bacteria and fungus from infecting the tree by precipitating out their enzymes and other protein exudates (Miele et al., 2020).

Alkaloids are a class of simple organic compounds originate in plants that have at least one nitrogen atom arranged in a ring structure (Bhambhani et al., 2021; Britannica, 2024). They were said to be the greatest active plant compounds with therapeutic significance (Heinrich et al., 2021). The result clearly indicated that the maximum amount of

alkaloid (296.8±0.536 mg/g) was reported in bark and least amount of (26.40 ± 0.126 mg/g) was observed in seeds.

In vitro antioxidant activity of selected parts of Psidium guajava L.

Aerobes depend on oxygen because it aids as a terminal electron acceptor during respiration, the process that produces the majority of their energy (Tokunou et al., 2022). On the other hand, a variety of illnesses in the human body are brought on by free radicals generated during the metabolic process (Pham-Huy et al., 2008; Gupta et al., 2014; Engwa et al., 2022).

Table 5 displayed the IC_{50} values for the in vitro antioxidant achievement of DPPH and ABTS, whereas Figures 4 and 5 depict the radial scavenging activities of *Psidium guajava* L. plant parts for DPPH and ABTS, respectively, in various volumes with percentages.

To evaluate the antioxidant potential of plant extracts, radical scavenging is a recognized method. However, it is important to avoid the negative

Table 5: IC_{50} values of DPPH and ABTS activity of *Psidium guajava* L.

Plant parts	DPPH assay (mg/ml)	ABTS assay (mg/ml)
Bark	57.61±0.153	431.09±0.224
Leaf	19.17±0.258	356.64±0.156
Fruit	87.40±0.526	1101.50±0.325
Seed	130.67±0.865	2096.23±0.521

Mean ± Standard Deviation; Means are significantly different ($P < 0.05$)

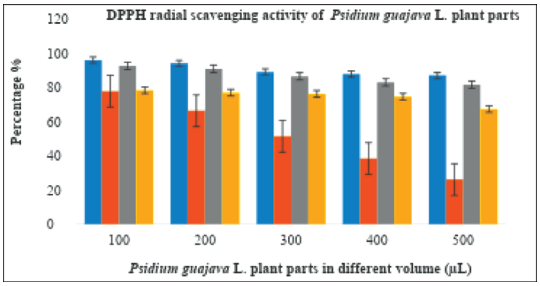

Figure 4. DPPH radial scavenging activity of *Psidium guajava* L. plant parts

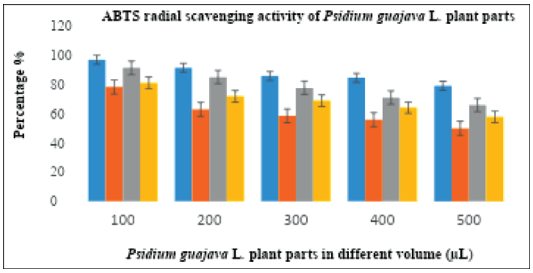

Figure 5. ABTS radial scavenging activity of *Psidium guajava* L. plant parts

consequences of free radical scavenging (Baliyan et al., 2022). The DPPH decolorized by accepting an electron donated by an antioxidant (Chen *et al*., 2007). In the DPPH assay, violet color DPPH solution is reduced to yellow colored creation, diphenylpicyl hydrazine, by the addition of the extract in a concentration dependent manner (Rahman *et al*., 2015).

Parts of the *Psidium guajava* L. plant showed an increase in their ability to scavenge free radicals (DPPH) in a concentration-dependent way. The activity was assessed by the decrease in absorbance as the consequence of DPPH color alteration from purple to yellow. According to the results, the greatest radical scavenging activity (as characterized by the lowest IC50) was observed in *Psidium guajava* L. leaves (IC50: 19.17±0.258 mg/ml) followed by bark, fruit and seeds. hence, the lowest radical scavenging activity (as characterized by the highest IC50) was observed in seeds (IC50: 130.67±0.865 mg/ml). The antioxidant action of the guava leaf is much advanced than other parts (Chen and Yen, 2007).

In a typical peroxidative reaction, the peroxidase substrate ABTS is oxidized in the presence of H_2O_2 (Hui-Yin et al., 2007). In This study $ABTS^+$ radical was used to assess the possible free radical-scavenging actions of guava extracts. In ABTS values (%) of guava leaves was shown lowest IC50 value (356.64±0.156 mg/ml), seeds were shown highest IC50 value (96.063±0.358 mg/ml). Interestingly, leaves have shown the highest radical

scavenging activity out of other parts.

Overall, the results of these DPPH and ABTS experiments showed that the methanolic extracts of the various *Psidium guajava* L. components were used in the following descending order: Fruits > seeds > bark > leaves.

It is possible to separate quercetin, quercetin-3-O-glucopyranoside, and morin from leaves. These substances exhibit antioxidant properties. Quercetin has the ability to balance free radicals. It has a far higher reducing power than any other substance. It is thought to be the maximum potent and dynamic antioxidant existing in guava leaves (Nasser et al., 2018). In an advanced study, guava leaf crude polysaccharides were used to produce silver nanoparticles, which exhibited significant DPPH and ABTS radical scavenging activity (Wang et al., 2017). The occurrence of gallic acid, catechol, taxifolin, ellagic acid, ferulic acid, and several other phenolic compounds are accountable for the antioxidant potential of guava leaves (Chen et al., 2007).

Conclusion

As a consequence, this research will provide the scientific community and the general public with information about the phytochemical potentials of various components of Sri Lankan-grown guava and their antioxidant effects. This research can be used by the scientific community to conduct more studies. The best parts to use in functional medicine for medical purposes may be those of the leaf and bark of this plant. This information can be very helpful in developing new biologically active products. Therefore, it could hypothetically be used as a nutraceutical constituent in the production of functional foods, making it an acceptable substitute for the treatment of a assortment of non-communicable ailments. It is also used to produce ready-to-use functional goods and nutraceuticals. Therefore, plant leaves, bark and fruit parts of *Psidium guajava* L. can be used as monotherapy adjunctive therapy in disease treatment in traditional medicine.

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