

HPLC-Based Quantitative Phytochemical Analysis of Aqueous and Ethanol Leaf Extracts of *Psidium guajava* L.

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Abstract

Psidium guajava (guava) leaves are widely used in traditional medicine, but comparative evidence on solvent-dependent differences in their phytochemical composition remains limited. This study compared the quantitative phytochemical profiles of aqueous and ethanol extracts of *P. guajava* leaves. Leaves collected from Wukari Local Government Area, Taraba State, Nigeria, were shade-dried, powdered, and separately macerated in 95% ethanol and distilled water for 72 hours. Individual bioactive compounds were analysed using high-performance liquid chromatography (HPLC) with diode-array detection. Both extracts tested positive for flavonoids, tannins, saponins, alkaloids, terpenoids, phenolics, and steroids. HPLC analysis identified quercetin, chlorogenic acid, vanillic acid, and caffeine in the ethanol extract, with caffeine constituting the most abundant identified compound at 9.4% of the total quantified content. The aqueous extract contained ellagic acid, luteolin, chrysin, pelargonidin, campesterol, stigmasterol, and vanillic acid, with ellagic acid predominating at 48.8% of the total quantified content. The ethanol extract exhibited higher quercetin and total phenolic contents, whereas the aqueous extract showed higher tannin and saponin contents. These findings indicate distinct solvent-dependent phytochemical profiles and provide comparative baseline data to guide extract selection for subsequent pharmacological evaluation, including investigations of potential antidiarrhoeal, haematoprotective, nephroprotective, and hepatoprotective applications.

Keywords: *Psidium guajava*; Phytochemical Profiling; High-Performance Liquid Chromatography; Extraction Solvent; Ellagic Acid

INTRODUCTION

Psidium guajava L. (guava), family Myrtaceae, is a widely distributed tropical shrub whose leaves have long been used in traditional medicine for the management of gastrointestinal disturbances, including diarrhea and dysentery (Naseer *et al.*, 2018; Dange *et al.*, 2020). Phytochemical investigations of guava leaves have repeatedly demonstrated that they are rich in bioactive secondary metabolites, including flavonoids, tannins, saponins, alkaloids, and diverse phenolic compounds (Möwes *et al.*, 2025). The leaves additionally contain appreciable concentrations of triterpenoids such as crategolic, guajavolic, oleanolic, and ursolic acids, together with essential oils composed of β -sitosterol, β -bisabolene, aromadendrene, β -selinene, β -caryophyllene, guajaverine, nerolidol, and selin-11-en-4 α -ol, which collectively contribute to the plant's broad therapeutic potential (Joseph and Priya, 2011).

Among the flavonoids reported in guava leaves, quercetin and quercetin-3-O- α -L-arabinopyranoside (guajaverin) have attracted particular attention. Quercetin exerts a spasmolytic effect on intestinal smooth muscle by modulating calcium-dependent contractile mechanisms (Chomentowski *et al.*, 2025), while guajaverin possesses notable antiplaque activity mediated through inhibition of *Streptococcus mutans* growth (Prabu *et al.*, 2006). Tannins contribute astringent, antisecretory, and antimicrobial properties (Cziple *et al.*, 2022; Nata'ala *et al.*, 2019), saponins contribute antimicrobial, anti-inflammatory, and antisecretory activity (Al Saiqali *et al.*, 2025; Ferede *et al.*, 2021), and alkaloids exert antispasmodic and analgesic effects broadly comparable to opioid antidiarrheal agents such as loperamide (Chan, 2008; Omojate Godstime *et al.*, 2014).

The choice of extraction solvent is known to strongly influence both the yield and the class of phytochemicals recovered from plant material. Ethanol, being of intermediate polarity, is generally more efficient at extracting flavonoid aglycones and glycosides and other medium-polarity phenolics, whereas water preferentially extracts highly polar constituents such as tannins and certain saponins (Seo *et al.*, 2014; Mehmood *et al.*, 2022; Timilsena *et al.*, 2023). Despite the substantial body of literature describing the phytochemistry of *P. guajava*, comparative quantitative data on how aqueous versus ethanol

extraction affects the specific bioactive compounds recovered from the leaves remain limited, particularly using chromatographic methods capable of resolving and quantifying individual constituents rather than crude phytochemical classes.

This study was therefore designed to qualitatively screen and quantitatively profile, by High Performance Liquid Chromatography (HPLC), the phytochemical constituents of aqueous and ethanol leaf extracts of *P. guajava*, in order to generate comparative baseline data that can guide the selection of the more suitable extract for downstream pharmacological evaluation.

MATERIALS AND METHODS

Collection and Authentication of Plant Material

Healthy, fully developed leaves of *Psidium guajava* were harvested from naturally growing plants in a pollution-free area of Wukari Local Government Area, Taraba State, Nigeria.

Preparation of Plant Material

The leaves were rinsed thoroughly with distilled water to remove adhering dust and debris, then shade-dried under ambient laboratory conditions (25–28 °C) for 7–10 days. The dried leaves were pulverized into a fine powder using a mechanical grinder, sieved through a 300 µm mesh, and stored in airtight amber containers at 4 °C until extraction.

Preparation of Ethanol and Aqueous Extracts

For ethanol extraction, 200 g of powdered leaves was macerated in 800 mL of 95% ethanol in a tightly stoppered conical flask at room temperature for 72 hours, with intermittent agitation every 6 hours. The mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure using a rotary evaporator (BUCHI B-100HB) maintained at 40–45 °C. The concentrate was dried completely under ambient conditions and stored in a desiccator at 4 °C.

For aqueous extraction, 200 g of powdered leaves was macerated in 800 mL of distilled water at room temperature for 72 hours with periodic agitation. The extract was filtered through Whatman No. 1 filter paper and the filtrate concentrated by freeze-drying or by evaporation in a water bath at 60 °C. The dried aqueous extract was similarly stored in a desiccator at 4 °C until analysis.

Quantitative Phytochemical Analysis (HPLC)

Quantitative estimation of individual phytochemicals (including quercetin, gallic acid, catechin, and other phenolic compounds) was performed by High Performance Liquid Chromatography using an Agilent 1260 Infinity II system fitted with a C18 reverse-phase column (250 mm × 4.6 mm, 5 µm particle size) and a diode array detector (DAD). The mobile phase consisted of 0.1% formic acid in water (solvent A) and ethanol (solvent B) delivered at a flow rate of 1.0 mL/min, with an injection volume of 20 µL. Detection was performed at 280 nm for phenolic compounds and 370 nm for flavonoids. Standard calibration curves were prepared using authentic reference compounds, and results were expressed as milligrams of compound per gram of extract, and as the amount (mg) and relative proportion (%) of each identified peak within the total quantified chromatographic profile.

Data Analysis

Quantitative data are presented descriptively as amount (mg), percentage of total quantified content, and retention time for each identified peak.

RESULTS

HPLC Profile of the Ethanol Extract

HPLC analysis of the ethanol leaf extract resolved several chromatographic peaks, of which four were identified as quercetin, chlorogenic acid, vanillic acid, and caffeine (Table 1; Figure 1). Caffeine was the most abundant identified compound (35.688 mg; 9.4% of total quantified content), followed by vanillic acid (22.834 mg; 6.0%), chlorogenic acid (4.075 mg; 1.1%), and quercetin (0.207 mg; 0.1%). Several additional, unidentified peaks were detected, indicating that the extract contains further phytochemical constituents beyond those matched to reference standards. The total quantified phytochemical content of the ethanol extract was 379.252 mg.

Table 1. HPLC phytochemical profile of the ethanol leaf extract of *Psidium guajava*

S/N	Retention Time (min)	Response	Amount (mg)	Amount (%)	Compound
1	0.213	2.683	2.683	0.7	Unidentified
2	0.517	225.852	225.852	59.6	Unidentified
3	1.132	18.782	18.782	5.0	Unidentified
4	1.617	13.578	13.578	3.6	Unidentified
5	1.832	12.326	0.207	0.1	Quercetin
6	2.480	70.951	4.075	1.1	Chlorogenic acid
7	2.793	55.551	55.551	14.6	Unidentified
8	3.825	171.258	22.834	6.0	Vanillic acid
9	6.208	735.170	35.688	9.4	Caffeine
Total			379.252	100.0	

HPLC Profile of the Aqueous Extract

HPLC analysis of the aqueous leaf extract identified seven bioactive compounds: ellagic acid, luteolin, chrysin, pelargonidin, campesterol, vanillic acid, and stigmasterol (Table 2; Figure 2). Ellagic acid was the predominant identified compound, accounting for 373,228.381 mg (48.8%) of the total identified content, followed by luteolin (183,810.316 mg; 24.0%), chrysin (121,057.503 mg; 15.8%), pelargonidin (65,882.269 mg; 8.6%), campesterol (10,928.833 mg; 1.4%), vanillic acid (2,160.258 mg; 0.3%), and stigmasterol (1,598.060 mg; 0.2%). Several unidentified peaks were also detected. The total quantified phytochemical content of the aqueous extract was 765,517.529 mg.

Table 2. HPLC phytochemical profile of the aqueous leaf extract of *Psidium guajava*

S/N	Retention Time (min)	Response	Amount (mg)	Amount (%)	Compound
1	0.552	0.284	0.284	0.0	Unidentified
2	2.825	189.116	189.116	0.0	Unidentified
3	3.307	1341.613	1341.613	0.2	Unidentified
4	3.950	16201.936	2160.258	0.3	Vanillic acid
5	5.435	398110.274	373228.381	48.8	Ellagic acid
6	6.770	2569.937	2569.937	0.3	Unidentified
7	12.855	2279762.895	183810.316	24.0	Luteolin
8	14.787	1655.142	1655.142	0.2	Unidentified
9	16.412	224041.079	10928.833	1.4	Campesterol
10	19.637	169480.505	121057.503	15.8	Chrysin
11	21.278	148235.106	65882.269	8.6	Pelargonidin
12	25.517	496.262	496.262	0.1	Unidentified
13	27.285	295.981	295.981	0.0	Unidentified
14	27.578	266.640	1598.060	0.2	Stigmasterol
15	28.823	11679.582	36.932	0.0	Unidentified
Total			765517.529	100.0	

DISCUSSION

The quantitative HPLC analysis revealed marked differences between the two extracts. The ethanol extract contained higher concentrations of quercetin and total phenolics, consistent with the well-documented efficiency of ethanol, a solvent of intermediate polarity, in solubilizing flavonoid aglycones and glycosides and other medium-polarity phenolic compounds (Seo *et al.*, 2014; Mehmood *et al.*, 2022; Nantitanon and Okonogi, 2012). By contrast, the aqueous extract contained higher levels of tannins and saponins, reflecting the highly polar nature of these compounds and their preferential extraction into water (Timilsena *et al.*, 2023). Tannins are known to precipitate proteins in the intestinal mucosa, reducing secretion and motility (Cziple *et al.*, 2022), while saponins contribute antimicrobial and anti-inflammatory activity (Al Saiqali *et al.*, 2025; Ferede *et al.*, 2021).

The specific compounds resolved by HPLC further differentiate the two extracts. Ellagic acid, luteolin, chrysin, pelargonidin, campesterol, and stigmasterol were identified in the aqueous extract, while quercetin, chlorogenic acid, vanillic acid, and caffeine were identified in the ethanol extract. Consistent with a comprehensive review that identified approximately 27 antidiarrheal or antibacterial compounds from *P. guajava*, including benzophenone glycosides, terpenes, polysaccharides, phenols, and flavonoids (Liu *et al.*, 2024), the phytochemical mixtures recovered here reflect the broader chemical diversity of this species. The predominance of ellagic acid (48.8% of total identified content) in the aqueous extract is notable, as ellagic acid is a potent antioxidant with demonstrated antimicrobial activity against enteric pathogens (Ahamad and Ansari, 2022; Huynh *et al.*, 2025), while the high proportions of luteolin and chrysin further support the therapeutic potential of the aqueous extraction route. In the ethanol extract, vanillic acid and chlorogenic acid are phenolic compounds with well-documented antioxidant and anti-inflammatory activity (Díaz-de-Cerio *et al.*, 2017; Huynh *et al.*, 2025), although caffeine, the most abundant identified compound in this extract, is not typically associated with antidiarrheal activity.

The presence of numerous unidentified chromatographic peaks in both extracts indicates that additional, uncharacterized phytochemicals remain to be resolved, and suggests that the overall pharmacological activity of *P. guajava* leaf extracts likely reflects synergistic interactions among multiple constituents rather than the action of any single

bioactive compound, consistent with the concept of polypharmacology in herbal medicine (Chen *et al.*, 2022; Vaou *et al.*, 2022).

CONCLUSION

Aqueous and ethanol *P. guajava* extracts contain flavonoids, tannins, saponins, alkaloids, terpenoids, phenolics, and steroids, but the ethanol extract is comparatively enriched in quercetin and total phenolics (including chlorogenic acid, vanillic acid, and caffeine), while the aqueous extract is comparatively enriched in tannins, saponins, and ellagic acid, luteolin, chrysin, and pelargonidin. These comparative data provide a phytochemical basis for interpreting the differential biological activities of the two extracts and support further isolation, characterization, and individual pharmacological evaluation of the major constituents identified, particularly quercetin and ellagic acid.

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