

Phytochemical and Antioxidant Profiling of n-Hexane and Ethyl Acetate Fractions of *Psidium guajava* Flowers

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Abstract : *The phytochemical composition and antioxidant activity of the n-hexane and ethyl acetate fractions of the methanol crude extract of Psidium guajava flowers were investigated. The plant material was collected from Emeyal I community, Bayelsa State, Nigeria, air-dried, pulverized, and macerated in methanol for 72 hours, followed by successive partitioning using n-hexane and ethyl acetate. The percentage yields of the fractions were 0.497% and 0.869% for the n-hexane and ethyl acetate fractions, respectively, indicating a higher abundance of moderately polar constituents. Qualitative phytochemical screening revealed the presence of steroids and glycosides in the n-hexane fraction, while alkaloids, flavonoids, and glycosides were detected in the ethyl acetate fraction. Thin-layer chromatography profiling showed multiple components with optimal resolution achieved using n-hexane: ethyl acetate (3:1) for the n-hexane fraction (six spots) and (1:3) for the ethyl acetate fraction (four spots), confirming the complexity of the phytochemical constituents.*

The antioxidant activity, evaluated using the DPPH radical scavenging assay at concentrations ranging from 50–400 µg/mL, showed a concentration-dependent increase in inhibition for both fractions. The ethyl acetate fraction exhibited higher radical scavenging activity, comparable to the standard ascorbic acid, indicating strong antioxidant potential.

These findings demonstrate that Psidium guajava flowers are a rich source of bioactive phytochemicals, particularly moderately polar compounds such as flavonoids, which significantly contribute to antioxidant activity. The study highlights the pharmacological potential of this underexplored plant part and

underscores the importance of solvent fractionation in enhancing bioactive compound recovery. Further studies are recommended to isolate, characterize, and evaluate the specific compounds responsible for the observed bioactivity for potential pharmaceutical and nutraceutical applications.

Keywords: *Psidium guajava* flowers, phytochemical profiling, antioxidant activity, DPPH assay, n-hexane fraction, ethyl acetate fraction, TLC analysis

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1.0 Introduction

Psidium guajava L. (guava) is a nutraceutical tropical plant widely distributed across Africa, Asia, and South America. Reports have shown that the plant has been used widely in traditional medicine for the treatment of diarrhoea, wounds, inflammation and microbial infections (Gutiérrez *et al.*, 2008; Biswas *et al.*, 2013). In addition to its traditional uses, various parts of the plant have been reported to possess significant pharmacological properties, including antioxidant, antimicrobial, and anti-inflammatory activities.

Phytochemicals are a diverse group of bioactive compounds found in natural sources such as plants. They have been responsible for the various bioactivities associated with both local and scientific medicinal claims of plants. These phytochemicals can be extracted from plant tissues via solvent fractionation, an important procedure in phytochemical investigation. This technique facilitates the separation of phytochemicals based on the principle of 'like dissolves like. Non-polar solvents like n-hexane will dissolve non-polar phytochemicals like the steroids, terpenoids, fatty acids, and a certain class of alkaloids (Harborne, 1998). Moderately polar solvents like ethyl acetate, dichloromethane and the more polar solvents like the alcohols, water will dissolve the more polar phytochemicals like the alkaloids, flavonoids, tannins, lignins, etc. These phytochemicals are pharmacologically significant compounds, notably for antioxidant, anti-inflammatory and antimicrobial characteristics. Previous studies on *Psidium guajava* have largely focused on the leaves and fruits, where compounds such as flavonoids, tannins, and phenolics have been identified as major contributors to its antioxidant activity.

Cellular and molecular damage have been attributed to oxidative stress arising from the imbalance between reactive oxygen species (ROS) catabolism and antioxidant defence systems. These ROS, such as the hydroxyl radicals, superoxide anions and hydrogen peroxide, have been linked to the pathogenesis of a range of severe diseases such as cardiovascular disorder, neurodegenerative conditions, cancer and diabetes (Pham-Huy *et al.*, 2008; Lobo *et al.*, 2010). Consequently, there is growing need in research and development to further identify antioxidants that are plant based capable of mitigating oxidative stress. This has intensified the search for safe, effective, and naturally derived antioxidants from plant sources.

The plant kingdom represents a broad spectrum of bioactive candidates ranging from alkaloids, flavonoids, terpenoids, steroids, tanins, lignins and phenolics with established profound physiological characteristics (Cowan, 1999; Wink, 2015). These bioactive candidates of plant origins, collectively known as phytochemicals, often act as free radical scavengers, metal ions chelators and modulators of enzymatic antioxidant systems (Pisoschi & Pop, 2015).

The present study is designed to investigate the qualitative phytochemical composition and the antioxidant activity of the n-hexane and the ethyl acetate fractions of the methanol crude of extract of *Psidium guajava* flowers, thus contributing to scientific knowledge on the medicinal potential of the plant under study. The leaves and other parts, like the fruits of *P. guajava*, have been studied extensively; however, limited attention has been given to its floral parts, particularly regarding their phytochemical composition and antioxidant potential when fractionated into non-polar and moderately polar solvents.

The findings from this study are expected to provide valuable insight into the potential of *Psidium guajava* flowers as a source of natural antioxidants and bioactive compounds, thereby supporting their possible applications in pharmaceutical and nutraceutical development.

2.0 Materials and Methods

2.1 Materials

2.1.1 Equipments and Apparatus

Analytical balance (Mettler JA 3003A), Rotary evaporator (RE100-S), pre-coated TLC plates, separating funnel, capillary tubes and other glassware.

2.1.2 Reagents

All reagents used in this study were of analytical grade and included: methanol, n-hexane, ethyl acetate, chloroform, sulphuric acid, hydrochloric acid, ferric chloride, bromine water, Hager's reagent, Molisch's



reagent, olive oil, sodium hydroxide, and distilled water.

2.2 Methods

2.2.1 Collection of Plant Material

The flower part of *P. guajava* was collected from a secondary forest in Emeyal community in Ogbia Local Government Area of Bayelsa State, Nigeria. The plant part was identified at the Department of Biology, Federal University Otuoke, Bayelsa State, Nigeria. A voucher specimen was prepared and deposited in the herbarium for future reference.

The collected flowers were shade-dried to constant weight, pulverized using a mechanical blender, and stored before extraction.

2.2.2 Extraction and Fractionation

About 23.928 g of the dried blended floral part was macerated in methanol for 72 hrs. The methanol extract was filtered, concentrated and successively partitioned using n-hexane, followed by ethyl acetate using a separatory funnel

The n-hexane and ethyl acetate fractions were then concentrated under vacuum with the aid of a rotary evaporator. The percentage yield was calculated using equation 1

$$\%yeild = \frac{\text{Weight fraction}}{\text{Initial weight of the flower}} \times \frac{100}{1} \quad (1)$$

2.3 Qualitative Phytochemical Test

Test for Alkaloids

To 2 mL of the fraction, a few drops of Hager's reagent (saturated solution of picric acid) were added. The formation of a yellow precipitate indicated the presence of alkaloids.

Test for Flavonoids

To 1 mL of the fraction, 10% lead acetate solution was added. The appearance of a yellow coloration indicated the presence of flavonoids.

Test for Steroids

To 50 mg of the fraction, 2 mL of chloroform and 3 mL of concentrated sulphuric acid were added carefully. The formation of a reddish-

brown ring at the interface indicated the presence of steroids.

Test for Tannins

To the fraction, 10 mL of bromine water was added. Decolourization of the bromine water indicated the presence of tannins.

Test for Saponins

To 200 mg of the fraction, 5 mL of distilled water and a few drops of olive oil were added. The mixture was shaken vigorously and allowed to stand. The formation of a stable, persistent froth indicated the presence of saponins.

Test for Glycosides

To 500 mg of the fraction, 2% hydrochloric acid was added and the mixture was heated in a water bath for 15 minutes. The solution was cooled and filtered, and the filtrate was neutralized with sodium hydroxide. To 2 mL of the neutralized filtrate, 3 drops of Molisch's reagent were added, followed by careful addition of 1 mL of concentrated sulphuric acid along the side of the test tube. The formation of a purple or violet ring indicated the presence of glycosides.

Thin Layer Chromatography (TLC) Profiling

One hundred milligrams (100 mg) of each fraction were dissolved separately in 0.5 mL of the appropriate solvent (n-hexane for the n-hexane fraction and ethyl acetate for the ethyl acetate fraction). Each solution was spotted onto pre-coated TLC plates and developed using different solvent systems: n-hexane:ethyl acetate (3:0, 3:1, 3:2) for the n-hexane fraction, and n-hexane:ethyl acetate (0:3, 1:3, 2:3) for the ethyl acetate fraction.

2.4 Antioxidant Profiling

2.4.1 -Diphenyl-1-picrylhydrazine (DPPH) Scavenging Activity

DPPH Radical Scavenging Activity

The DPPH radical scavenging activity of the fractions was determined using the method described by Odokwo and Eze (2024), with ascorbic acid (vitamin C) used as the standard. Briefly, 1.0 mL of each fraction at varying concentrations (50–400 µg/mL) was mixed



with 1.0 mL of 0.1 mM DPPH solution prepared in methanol. The reaction mixtures were incubated in the dark for 30 minutes, after which the absorbance was measured at 517 nm using a spectrophotometer.

The percentage inhibition of DPPH radicals was calculated using equation 2

$$\%Inhibition = \frac{A_c - A_s}{A_c} \times \frac{100}{1} \quad (2)$$

where A_c is the absorbance of the control (blank) sample, and A_s is the absorbance of the sample.

Statistical Analysis

All experiments were conducted in triplicate, and the results were expressed as mean \pm standard deviation.

3.0 Results and Discussion

The extraction and solvent partitioning of the methanol crude extract of *Psidium guajava* flowers yielded fractions with varying mass recoveries, as presented in Table 1. The ethyl acetate fraction exhibited a higher percentage yield (0.869%) compared to the n-hexane fraction (0.497%), indicating a predominance of moderately polar constituents within the plant matrix. This observation is consistent with the general phytochemical distribution in plant tissues, where phenolic and flavonoid compounds—typically of moderate polarity—are more abundant than non-polar constituents such as lipids and waxes. The higher recovery in the ethyl acetate fraction therefore, suggests that the floral part of *Psidium guajava* is a richer source of semi-polar bioactive compounds, which are often associated with significant biological activities.

Table 1: Yield of Fractions

Material	Weight (g)	%Yield
Floral	23.938 g	
n-hexane fraction	0.119	0.497
EtOAc fraction	0.208	0.869

*EtOAc – ethyl acetate



The qualitative phytochemical screening results presented in Table 2 further support this distribution pattern. Alkaloids and flavonoids were detected exclusively in the ethyl acetate fraction, whereas steroids were confined to the n-hexane fraction, and glycosides were present in both fractions. The absence of tannins and saponins in both fractions may indicate either their low abundance in the floral matrix or limited extraction under the employed conditions. The selective partitioning of alkaloids and flavonoids into the ethyl acetate fraction reflects their intermediate polarity, while the presence of steroids in the n-hexane fraction aligns with their hydrophobic nature. These findings are in agreement with established phytochemical extraction principles and reinforce the efficiency of solvent fractionation in separating compounds based on polarity.

Table 2: Qualitative Phytochemical Analysis of n-Hexane and Ethyl Acetate Fractions

S/Nos	Phytochemical	Fraction	
		N-Hexane	EtOAc
1.	Alkaloids	-	+
2.	Flavonoids	-	+
3.	Steroids	+	-
4.	Tannins	-	-
5.	Saponins	-	-
6.	Glycosides	+	+

*+: present, -: absent

From a pharmacological perspective, the detected phytochemical classes have well-documented bioactivities. Alkaloids are known to exhibit antimicrobial and anticancer properties (Cushnie *et al.*, 2014), while flavonoids are widely recognized for their potent antioxidant capacity due to their ability to donate hydrogen atoms and stabilize free radicals (Panche *et al.*, 2016). Steroidal compounds have been associated with anti-inflammatory and immunomodulatory effects (Rhen & Cidowski, 2005), whereas glycosides

encompass a broad class of compounds with diverse therapeutic potentials, including cardioprotective, neuroprotective, and antidiabetic activities (Man *et al.*, 2010; Kim, 2012; Chatsudhipon & Muanprasat, 2009). The coexistence of these phytochemical classes in the investigated fractions highlights the pharmacological relevance of the floral part of *Psidium guajava*.

The thin layer chromatography (TLC) profiling results summarized in Table 3 revealed multiple spots across the different solvent systems for both fractions, indicating the presence of multiple phytochemical constituents. The n-hexane fraction exhibited a maximum of six distinct spots when developed in the n-hexane:ethyl acetate (3:1) solvent system, while the ethyl acetate fraction showed optimal resolution with four spots in the n-hexane:ethyl acetate (1:3) system. The variation in the number of resolved spots and corresponding R_f values across solvent systems reflects differences in compound polarity and interaction with the stationary phase. The higher number of spots observed in the n-hexane fraction suggests a greater diversity of non-polar constituents, whereas the fewer but distinct spots in the ethyl acetate fraction may indicate a relatively more selective composition of moderately polar

compounds. The identification of optimal solvent systems is critical for compound separation and provides a basis for future isolation and purification studies. Overall, the TLC results confirm the chemical complexity of both fractions and validate the effectiveness of the chosen solvent systems in resolving their constituents.

The antioxidant activity of the fractions, as illustrated in Figure 1, demonstrated a concentration-dependent increase in DPPH radical scavenging activity for both the n-hexane and ethyl acetate fractions. Notably, the ethyl acetate fraction exhibited superior antioxidant activity, approaching that of the standard ascorbic acid at higher concentrations. This enhanced activity can be attributed to the presence of flavonoids and other phenolic compounds detected in this fraction, which are known to possess strong radical scavenging properties through electron or hydrogen donation mechanisms. In contrast, the n-hexane fraction, although less active, still displayed appreciable antioxidant activity, likely due to the presence of non-polar antioxidant constituents such as steroids and terpenoids, which have been reported to contribute to oxidative stress mitigation through alternative mechanisms.

Table 3: TLC Analysis of n-Hexane and Ethyl Acetate Fractions

S/Nos.	n-Hexane Fraction			EtOAc Fraction		
	No. of Spots	R _f	Solvent System	No. of Spots	R _f	Solvent System
1.	5	0.08, 0.26, 0.44, 0.51, 0.95	100% n-hexane (3 ml)	2	0.10, 0.91	100% EtOAc
2.	6	0.17, 0.33, 0.46, 0.62, 0.73, 0.93	n-hexane: EtOAc (3:1)	4	0.33, 0.62, 0.84, 0.91	n-hexane: EtOAc (1:3)
3.	4	0.09, 0.61, 0.75, 0.90	n-hexane: EtOAc (3:2)	3	0.68, 0.81, 0.95	n-hexane: EtOAc (2:3)

** EtOAc = Ethyl Acetate



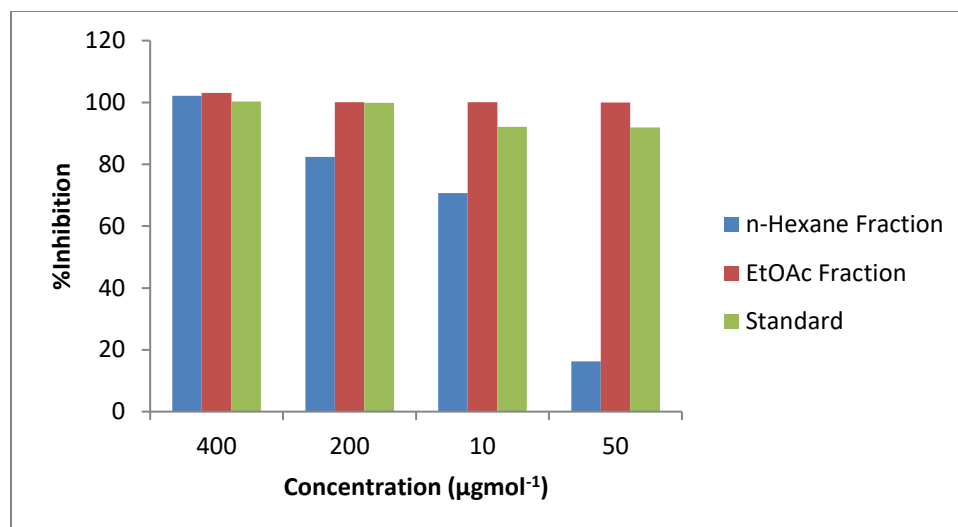


Fig. 1: DPPH Scavenging Activity of the *N*-Hexane and Ethyl Acetate Fractions

Comparatively, the higher antioxidant capacity of the ethyl acetate fraction aligns with previous studies on *Psidium guajava*, where polar and semi-polar extracts consistently demonstrated stronger antioxidant activities than non-polar extracts (Thaipong *et al.*, 2006; Qian and Nihorimbere, 2004). This trend underscores the significant role of phenolic and flavonoid compounds in antioxidant defense. The results obtained in this study therefore corroborate existing literature while specifically highlighting the underexplored floral part of the plant as a viable source of antioxidant agents.

From a technical standpoint, the findings suggest that solvent polarity plays a crucial role not only in extraction efficiency but also in determining the biological activity of plant extracts. The superior performance of the ethyl acetate fraction indicates its potential suitability for further phytochemical isolation and development of antioxidant formulations. Additionally, the demonstrated antioxidant activity of both fractions supports their possible application in pharmaceutical, nutraceutical, and functional food industries as natural alternatives to synthetic antioxidants.

4.0 Conclusion

This study demonstrates that both the n-hexane and ethyl acetate fractions of the methanol

crude extract of *Psidium guajava* flowers contain diverse bioactive phytochemicals with appreciable antioxidant activity. The higher activity observed in the ethyl acetate fraction highlights the significance of moderately polar constituents, particularly flavonoids and related compounds, in contributing to the overall radical scavenging potential. These findings confirm that the floral part of *Psidium guajava*, which is relatively underexplored compared to its leaves and fruits, possesses considerable pharmacological potential.

The results further underscore the importance of solvent fractionation in enhancing the recovery and activity of bioactive compounds. Given the demonstrated antioxidant properties, the floral extract represents a promising source of natural antioxidants with potential applications in pharmaceutical and nutraceutical development. Consequently, further studies are warranted to isolate, purify, and structurally characterize the specific compounds responsible for the observed bioactivity, as well as to evaluate their mechanisms of action and therapeutic relevance.

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Declarations

Conflict of Interest

The authors declared no conflict of interest

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Ethical consideration

Not applicable

Data Availability.



All data generated or analysed during this study are included in this published article[and its supplementary information files.

Author Contributions

Edet O. Odokwo conceived and designed the study, supervised the research, performed data analysis, and led manuscript drafting and

revision. Martha S. Onifade conducted laboratory experiments, including extraction, fractionation, phytochemical screening, TLC, and antioxidant assays, and contributed to data interpretation and manuscript preparation. Both authors reviewed and approved the final manuscript.

