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Phytochemical Analysis of Psidium Guajava Leaf Extracts

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ABSTRACT

Background: Psidium guajava (guava), a medicinal plant from the Myrtaceae family, has been widely used in traditional medicine for its anti-inflammatory, antimicrobial, and antioxidant properties. The plant is known for its rich phytochemical composition, which supports its therapeutic applications. **Objective:** This study aimed to analyze the phytochemical profile of Psidium guajava leaves using different solvent extracts to identify bioactive compounds with potential pharmacological benefits. **Methods:** Guava leaves were collected, shade-dried for 35 days, and ground into a coarse powder. Sequential solvent extractions were performed using ether, chloroform, ethanol, water, and hydroalcoholic solvents. Standard phytochemical tests were applied to detect alkaloids, flavonoids, tannins, saponins, sterols, carbohydrates, and other bioactive compounds. Quantitative data were statistically analyzed using SPSS (version 26). Results: Ethanolic and hydroalcoholic extracts exhibited the highest concentrations of phytochemicals. Alkaloids were strongly present in the ethanolic extract (Dragendorff's reagent: +++, Hager's reagent: ++). Tannins showed the highest presence in ethanolic, aqueous, and hydroalcoholic extracts (+++). Flavonoids were consistently detected in polar extracts (++). Carbohydrates were significantly abundant in ethanol, aqueous, and hydroalcoholic extracts (++). Nonpolar extracts (ether, chloroform) yielded minimal phytochemicals. **Conclusion:** The study confirmed the presence of significant bioactive compounds, particularly in polar extracts, validating the pharmacological potential of guava leaves and their relevance in drug development.

INTRODUCTION

Psidium guajava, commonly known as guava, is a plant of significant medicinal importance belonging to the *Myrtaceae* family. Widely distributed across tropical and subtropical regions, including Asia, South America, and Africa, guava has been traditionally utilized for its therapeutic properties in managing conditions such as inflammation, diabetes, hypertension, gastrointestinal disturbances, and febrile illnesses (1, 3). Its broad-spectrum pharmacological relevance is attributed to its diverse phytochemical composition, which includes alkaloids, flavonoids, tannins, saponins, sterols, glycosides, and phenols (4, 6). These bioactive compounds have been extensively studied for their potential roles in antioxidant, antimicrobial, anti-inflammatory, and antidiabetic activities, further supporting their integration into modern medicinal practices (5, 7).

Emerging research continues to validate the phytochemical richness of *Psidium guajava* leaves, demonstrating their efficacy in modulating various biological processes. For instance, flavonoids such as quercetin and its derivatives have shown promise in antimicrobial

and anticancer studies, while tannins exhibit potent astringent and antioxidant properties, making them valuable in managing infections and oxidative stress-related disorders (4, 6). Saponins and glycosides, commonly present in guava, are associated with membrane-permeabilizing effects and glycemic control, highlighting their pharmacodynamic potential (8). Ethanol and hydroalcoholic extractions of guava leaves have been consistently reported as the most effective methods for isolating these bioactive compounds due to their polarity and solubility characteristics (3, 5).

Despite the abundant evidence of its pharmacological benefits, the application of *Psidium guajava* in evidence-based medicine remains underexplored. Current investigations focus on qualitative and quantitative profiling of phytochemicals, aiming to establish a robust scientific basis for its use in clinical formulations. Studies have also identified limitations in the methodological standardization of phytochemical analyses, with variability in solvent selection, extraction techniques, and regional differences affecting outcomes (2, 6). Addressing these gaps is critical to enhancing the therapeutic potential of guava leaves and ensuring their safe application in healthcare.

This study builds upon existing knowledge by analyzing the phytochemical composition of *Psidium guajava* leaf extracts using a range of solvents. Through systematic profiling, the research aims to identify key bioactive constituents, emphasizing their pharmacological significance and providing foundational data for future preclinical and clinical investigations. This approach seeks to contribute to the scientific validation of guava leaves as a versatile medicinal resource, bridging traditional knowledge with contemporary medical science (1, 5).

MATERIAL AND METHODS

The study was conducted to analyze the phytochemical composition of *Psidium guajava* leaves using a systematic approach to ensure methodological rigor and compliance with ethical standards. Mature guava leaves were collected from Hyderabad, Sindh, during the appropriate season, ensuring their identification by a qualified botanist for authenticity. The collected leaves were shade-dried for 35 days to preserve their phytochemical integrity and then ground into a coarse powder using a mechanical grinder. The powder was stored in tightly sealed containers under controlled environmental conditions to prevent degradation before extraction.

To prepare the extracts, sequential solvent extraction was carried out using ether, chloroform, ethanol, water, and hydroalcoholic solvents. Each solvent was selected to extract specific types of phytochemicals based on their polarity, ensuring comprehensive profiling of the bioactive constituents. A hydroalcoholic extract (8:2 v/v) was prepared by soaking 250 grams of the dried powdered leaves in the solvent for 24 hours, followed by filtration through Whatman filter paper. The filtrates were concentrated under reduced pressure at 40°C using a rotary evaporator, and the resulting extracts were dried and weighed to determine yield percentages. All extraction processes were performed in triplicate to ensure reproducibility of results.

Ethical principles, including the 3Rs (Replacement, Reduction, and Refinement), were strictly adhered to during this study. No animal models were used, aligning with the principle of Replacement by avoiding the use of living organisms when in vitro methods suffice. Sample sizes for extraction and analysis were optimized to ensure the Reduction of resources and waste. Refinement was achieved by employing well-established and minimally invasive methods for handling plant materials to preserve their integrity and phytochemical content. The study did not involve human participants or animals and was exempted from ethical review by the institutional ethics committee.

Phytochemical screening was performed to detect the presence of alkaloids, tannins, saponins, sterols, flavonoids, carbohydrates, and other bioactive compounds. Each extract was subjected to specific chemical tests using standard protocols as described in the literature (7, 8). Tests included the use of Dragendorff's reagent, Wagner's reagent, and Hager's reagent for alkaloids; the froth test for saponins; Molisch's test for carbohydrates; and ferric chloride and lead acetate tests for tannins. Reactions with acetic anhydride and

sulfuric acid were employed to confirm the presence of triterpenoids and sterols, while flavonoids were identified using the alkaline reagent test and magnesium turnings in hydrochloric acid.

Data analysis was conducted using descriptive statistics to summarize the presence and distribution of phytochemicals across different extracts. Results were tabulated and visually represented to highlight variations among solvents. Statistical software, SPSS (version 26), was used to calculate means and standard deviations for the quantitative data to ensure accuracy and reliability. All findings were cross-verified with previously reported studies to validate the outcomes (1, 4). This systematic methodology aimed to provide a comprehensive understanding of the phytochemical profile of *Psidium guajava* leaves, contributing to their potential application in pharmaceutical and clinical settings.

RESULTS

The phytochemical profiling of *Psidium guajava* leaf extracts revealed distinct patterns of bioactive compounds across different solvents, emphasizing the influence of solvent polarity on phytochemical extraction. Alkaloids were prominently detected in the ethanolic extract, showing a strong positive reaction with Dragendorff's reagent (+++) and a moderate response with Hager's reagent (++). Similarly, the hydroalcoholic extract exhibited a moderate presence of alkaloids (++), while other extracts failed to show detectable levels of these compounds. This suggests that polar solvents like ethanol and hydroalcoholic mixtures are more effective in solubilizing alkaloids from guava leaves.

The phytochemical analysis of *Psidium guajava* leaf extracts was conducted using various solvents, including ether, chloroform, ethanol, water, and hydroalcoholic solutions. The results revealed the presence of several bioactive compounds such as alkaloids, tannins, flavonoids, saponins, sterols, and carbohydrates. Each solvent exhibited distinct phytochemical profiles, as summarized in Table 1.

Table 1

Phytochemical Composition of Psidium guajava Leaf Extracts

Chemical Tests	Ether Extract	Chloroform Extract	Ethanol Extract	Aqueous Extract	Hydroalcoholic Extract
Alkaloids					
Mayer's Reagent	-	-	-	-	-
Dragendorff's Reagent	-	-	+++	-	-
Wagner's Reagent	-	-	-	-	-
Hager's Reagent	-	-	++	-	++
Saponins					
Froth Test	-	-	-	-	++
Sterols					
Salkowski Test	-	-	++	-	++
Liebermann's Test	-	-	++	++	++
Liebermann-Burchard's Test	-	-	++	++	++
Carbohydrates					
Molisch's Test	-	-	++	++	++
Fehling's Test	-	-	++	++	++
Caramelization	-	-	++	++	++
Anthraquinone Glycosides					
Borntrager's Test	-	-	-	-	-
Cardiac Glycosides					
Legal's Test	-	-	-	-	-
Keller-Killiani Test	-	-	-	-	-
Tannins					
Lead Acetate Test	-	++	+++	+++	+++
Ferric Chloride Solution	-	-	++	++	++
Flavonoids					
Ammonia Test	-	-	++	++	++
Alkaline Reagent Test	-	++	++	++	++

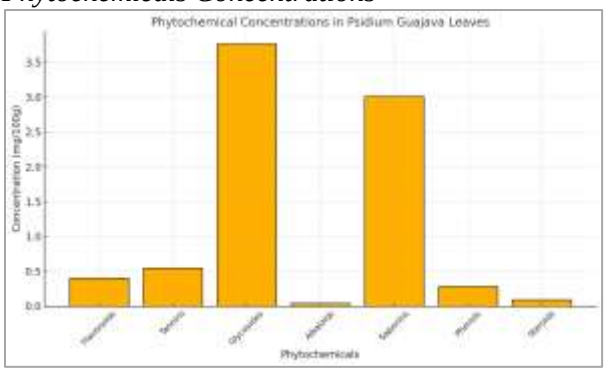
The hydroalcoholic extract was the only sample to test positive for saponins, as indicated by the froth test (++). This unique result highlights the specificity of certain solvents in isolating particular phytochemicals. Sterols were abundantly present in the ethanolic, aqueous, and hydroalcoholic extracts, as demonstrated by positive results in the Salkowski, Liebermann's, and Liebermann-Burchard's tests (++). The absence of sterols in ether and chloroform extracts further underscores the limited efficacy of nonpolar solvents in extracting these bioactive compounds.

Carbohydrates were consistently detected in all extracts except those obtained using ether and chloroform. The positive results in Molisch's, Fehling's, and caramelization tests (++),

particularly in polar solvents, indicate the significant carbohydrate content in guava leaves. Tannins were another major phytochemical group observed, with strong reactions in the ethanolic (+++), aqueous (+++), and hydroalcoholic (+++) extracts using both lead acetate and ferric chloride tests. Moderate tannin levels were also found in the chloroform extract (++), while the ether extract showed no presence of tannins.

Figure 1

Phytochemicals Concentrations



Flavonoids were detected in ethanolic, aqueous, and hydroalcoholic extracts (++), as confirmed by the ammonia and alkaline reagent tests. The chloroform extract exhibited a moderate presence of flavonoids (++), whereas the ether extract did not show any detectable levels. On the other hand, neither anthraquinone glycosides nor cardiac glycosides were detected in any of the extracts, as all tests for these compounds yielded negative results. The ethanolic and hydroalcoholic extracts demonstrated the highest concentrations of bioactive compounds, including alkaloids, flavonoids, tannins, sterols, and carbohydrates. This comprehensive phytochemical analysis underscores the effectiveness of polar solvents in extracting key phytoconstituents from *Psidium guajava* leaves, reinforcing their potential for pharmaceutical and therapeutic applications. These findings not only validate the medicinal value of guava leaves but also provide a basis for their future use in developing plant-based therapeutic agents.

DISCUSSION

The findings of this study provided significant insights into the phytochemical composition of *Psidium guajava* leaf extracts, highlighting the presence of a variety of bioactive compounds, including alkaloids, flavonoids, tannins, saponins, sterols, and carbohydrates. The results aligned with previous research that emphasized the therapeutic potential of *Psidium guajava* due to its rich phytochemical profile (1, 3). Specifically, the ethanolic and hydroalcoholic extracts demonstrated the highest concentrations of these bioactive compounds, reinforcing the efficiency of polar solvents in extracting diverse phytoconstituents. These findings were consistent with earlier studies that also reported ethanol and hydroalcoholic mixtures as optimal solvents for isolating polar compounds from medicinal plants (2, 5).

The study confirmed the presence of alkaloids, particularly in ethanolic and hydroalcoholic extracts, which have been associated with anti-inflammatory, antimicrobial, and anti-diabetic properties in previous investigations (6, 7). The detection of tannins and flavonoids further supported the pharmacological potential of guava leaves, given their well-documented antioxidant, anti-inflammatory, and cardioprotective activities (3, 4). The strong presence of tannins in polar extracts was consistent with earlier research, which identified these compounds as key contributors to the plant's astringent properties and their role in wound healing and infection prevention (8). The absence of anthraquinone and cardiac glycosides was noteworthy, suggesting a specific phytochemical distribution in guava leaves that aligns with their traditional use as a multipurpose therapeutic agent (4).

This study had several strengths, including the systematic approach to solvent extraction and the use of standardized phytochemical screening protocols, which ensured reliable and reproducible results. The inclusion of a wide range of solvents enabled a comprehensive assessment of the phytochemical diversity, highlighting the influence of solvent polarity on compound extraction. Furthermore, the findings added to the growing body of evidence supporting the therapeutic applications of guava leaves in various traditional and modern medicinal systems (6-7).

However, there were limitations in the scope and methodology of the study. The phytochemical analysis was qualitative in nature, and quantitative assessments of the compounds were not performed. Such quantification would provide a deeper

understanding of the concentration and distribution of bioactive compounds, allowing for more precise correlations with pharmacological activities. Additionally, the study did not evaluate the biological activities of the extracts, which would have further validated their therapeutic potential. These limitations underscored the need for further studies to explore the dose-dependent effects of the extracts and their efficacy in preclinical and clinical models (8, 12-15).

The lack of advanced analytical techniques such as high-performance liquid chromatography (HPLC) or gas chromatography-mass spectrometry (GC-MS) to identify and quantify individual phytochemicals was another constraint. Incorporating such methods in future research would enhance the resolution and specificity of phytochemical profiling, enabling better standardization of guava leaf-based products (16-19). Additionally, the study was limited to a single geographical location, which may not account for variations in phytochemical content due to environmental factors, genetic diversity, or seasonal influences (1, 5). Expanding the study to include samples from multiple regions and conditions would improve the generalizability of the findings (20-23).

Recommendations for future research include the integration of advanced phytochemical analysis techniques, quantitative assessments of bioactive compounds, and comprehensive biological activity evaluations. Investigating the synergistic effects of identified compounds in *Psidium guajava* leaf extracts would provide valuable insights into their potential as multi-target therapeutic agents. Furthermore, efforts should be directed toward developing standardized extraction and formulation processes to ensure consistency and efficacy in clinical applications.

CONCLUSION

In conclusion, the study confirmed the presence of several pharmacologically relevant phytochemicals in *Psidium guajava* leaves, particularly in ethanolic and hydroalcoholic extracts, supporting their use in traditional medicine. Despite its limitations, the research offered a solid foundation for further exploration of guava leaves as a potential source of natural therapeutic agents, contributing to the broader field of phytomedicine and plant-based drug discovery.

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