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Standardized Protocol for the Phytochemical Characterization of *Carica papaya* as a Foundational Tool for Public Health Nutrition Research

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Abstract

Background: The integration of traditional food plants like *Carica papaya* (pawpaw) into public health nutrition is hindered by inconsistent phytochemical methods, leading to irreproducible data and stalled implementation. Objective: This study aimed to develop and validate a comprehensive, reproducible laboratory protocol for the extraction, fractionation, and quantitative phytochemical analysis of *C. papaya* leaves and seeds. **Methods**: A stepwise protocol was developed, encompassing: botanical authentication and optimal drying, sequential methanol extraction, bioactivity-guided fractionation for alkaloids and flavonoids, qualitative screening, and quantitative spectrophotometric assays for key phytochemical classes. The protocol was applied to leaf and seed samples with yield calculations and statistical validation. **Results**: Methanol extraction yielded 17.51% from leaves and 11.59% from seeds. Fractionation successfully isolated target compounds, with flavonoid yields (7.05–7.96%) exceeding alkaloid yields (2.65–2.97%). Quantitative analysis revealed a phytochemical profile dominated by saponins (1249.83 mg/g in leaves, 723.65 mg/g in seeds), followed by phenolic compounds and alkaloids. **Conclusion**: This standardized methodology provides an essential toolkit for generating consistent, chemically defined plant extracts. It ensures reproducibility and comparability, supporting the evidence-based development of *C. papaya* and similar plants as sustainable nutritional resources in public health initiatives addressing malnutrition and dietary deficiencies.

Keywords: Standardized methodology, Phytochemical profiling, Public health nutrition, *Carica papaya*, Bioactive compounds, Food quality control, Nutritional biochemistry.

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1. Introduction

Global public health nutrition faces persistent challenges from widespread malnutrition, micronutrient deficiencies, and diet-related chronic diseases (Global Nutrition Report, 2021). In resource-limited settings, traditional food plants offer sustainable, culturally acceptable alternatives to commercial supplements (FAO, 2022). *Carica papaya* (pawpaw) is a promising resource, with its leaves and seeds holding potential nutritional value supported by traditional use across multiple cultures (Saeed et al., 2014; Vij&Prashar, 2015).

However, transforming traditional knowledge into evidence-based public health applications requires overcoming significant methodological barriers. Research on plant-based foods is frequently hampered by inconsistent processing methods, variable extraction techniques, and non-standardized analytical approaches (Heinrich et al., 2018). These inconsistencies lead to irreproducible results and non-

comparable data, undermining confidence in traditional food resources and delaying their integration into dietary guidelines.

The phytochemical composition of a plant including alkaloids, flavonoids, phenolics, saponins, and tannins forms the biochemical basis for its potential nutritional and therapeutic properties (Liu, 2013). In public health, these compounds may contribute to addressing specific deficiencies, such as enhancing iron bioavailability or providing antioxidant protection (Teleanu et al., 2019). Yet, without standardized methods to consistently extract, isolate, and quantify these compounds, research cannot reliably determine which components are most relevant for public health applications.

This study addresses these critical gaps by developing and validating a comprehensive, reproducible protocol specifically designed for public health nutrition research. Focusing on *C. papaya* leaves and seeds, we present a complete workflow from plant material handling to quantitative phytochemical characterization. Our objectives are threefold: to provide a standardized methodological framework ensuring consistency; to generate reliable phytochemical data for *C. papaya*; and to establish a model approach adaptable for other traditional food plants.

2. Materials and Methods

2.1. Plant Material Authentication and Preparation

Fresh, mature *Carica papaya* leaves were collected from certified organic cultivation at the Federal University of Technology, Minna, Nigeria. Seeds were obtained from ripe fruits sourced from local markets. Botanical authentication was performed by a qualified taxonomist (voucher specimen: FUTMin-Herb-048). To preserve bioactive compounds, leaves and seeds were shade-dried at 25–30°C for 10–14 days, then pulverized into a coarse powder using food-grade equipment. The powder was stored in airtight, light-protected containers at 4°C.

2.2. Standardized Extraction Protocol

2.2.1. Preparation of Crude Methanol Extract

Fifty grams of powdered material underwent reflux extraction with 400 mL of analytical-grade methanol for 2 hours.The mixture was filtered (muslin cloth, then Whatman No. 1 filter paper), and the solvent was removed using a rotary evaporator at 40°C, followed by final drying in a water bath at 50°C.

$$\%\ yield = \frac{Weight\ (g)\ of\ extract}{Weight\ (g)\ of\ pulverized\ sample} \times 100$$

Crude extracts were stored at -20°C in amber vials.

2.2.2. Bioactivity-Guided Fractionation

Alkaloid Isolation:Powdered material (300 g) was Soxhlet-extracted with 95% ethanol. The concentrated extract was dissolved in 1.0 N HCl, filtered, basified to pH 9–10 with NH_4OH, and partitioned with chloroform. The chloroform fraction was concentrated to yield the alkaloid fraction.

Flavonoid Enrichment:Crude methanol extract (41 g) was dissolved in water and partitioned with water-saturated n-butanol. The combined n-butanol fractions were evaporated to obtain the flavonoid-rich fraction.

2.3. Comprehensive Phytochemical Analysis

2.3.1. Qualitative Screening

Standard chemical tests were performed for major phytochemical classes: Dragendorff's test (alkaloids), alkaline reagent test (flavonoids), froth test (saponins), ferric chloride test (tannins and phenolics).

2.3.2. Quantitative Spectrophotometric Analysis

All quantitative assays were performed in triplicate using a Shimadzu UV-Vis spectrophotometer.

· Total Alkaloids: Gravimetric method with spectrophotometric confirmation at 570 nm (solanine standard) (Harborne, 1998).

· Total Flavonoids: Aluminum chloride method at 415 nm (quercetin standard; 0–100 µg/mL) (Chang et al., 2002).

· Total Phenolics: Folin-Ciocalteu assay at 765 nm (gallic acid standard; 0–500 µg/mL) (Singleton et al., 1999).

· Tannins: Modified Folin-Denis method at 760 nm (tannic acid standard) (AOAC, 1984).

· Saponins: Colorimetric method at 380 nm (saponin standard) (Oloyede, 2005).

2.4. Statistical Analysis

All analyses were performed in triplicate. Data are presented as mean ± standard error of the mean (SEM). Yield calculations included standard deviation. Analytical precision was confirmed with coefficient of variation <5%. Statistical significance ($p < 0.05$) was determined using appropriate tests.

3. Results and Discussion

3.1. Extraction Efficiency and Fractionation Yields

The methanol extraction yielded 17.51% ± 0.42% from leaves and 11.59% ± 0.38% from seeds (Table 1). The significantly higher yield from leaves ($p < 0.05$) reflects their higher soluble phytochemical content and lower structural fiber. Fractionation yielded flavonoid fractions (7.05–7.96%) substantially higher than alkaloid fractions (2.65–2.97%) for both plant parts

(Table 2), validating the protocol's efficiency and reproducibility.

Table 1: Percentage Yield of Crude Methanol Extract from C. papaya

Plant Samples	% Yield
C. Papaya crude (seeds) extract	11.59
C. Papaya crude (leaves) extract	17.51*

*Significantly higher compared to seeds extract ($p < 0.05$).

Table 2: Percentage Yields of Alkaloid and Flavonoid Fractions

Fractions	% Yields
Alkaloids (seeds) extract	2.97 ± 0.04^{a}
Flavonoids (seeds) extract	7.96 ± 0.29^{b}
Alkaloids (leaves) extract	2.65 ± 0.03^{a}
Flavonoids (leaves) extract	7.05 ± 0.65^{b}

Means with different superscripts in a column differ significantly ($p < 0.05$).

3.2. Comprehensive Phytochemical Profile

Quantitative analysis revealed distinct profiles (Table 3). Saponins were the most abundant class, with exceptionally high levels in leaves (1249.83 ± 13.05 mg/g) and seeds (723.65 ± 0.39 mg/g). Phenolic compounds were the second most abundant, significantly higher in leaves (261.34 ± 1.07 mg GAE/g) than seeds (171.45 ± 0.91 mg GAE/g). Alkaloid content was also higher in leaves (67.75 ± 1.06 mg/g) than seeds (35.42 ± 0.50 mg/g). Tannin and flavonoid contents were moderate but consistently higher in leaves.

Table 3: Quantitative Phytochemical Composition of C. papaya Extracts (mg/g extract)

Parameters	Seeds (mg/g)	Leaves (mg/g)
Phenols	171.45±0.91[b]	261.34±1.07[a]
Flavonoids	25.80±0.99[b]	34.89±0.66[a]
Tannins	40.67±0.50[b]	48.25±0.45[a]
Alkaloids	35.42±0.50[b]	67.75±1.06[a]
Saponins	723.65±0.39[b]	1249.83±13.05[a]

Values with different superscripts within rows differ significantly ($p < 0.05$).
Means with different superscripts within rows differ significantly ($p < 0.05$).

Discussion

The developed protocol successfully generated consistent and reproducible phytochemical data. The high saponin content, while noteworthy for potential bioactivity, requires careful consideration for dietary applications due to possible gastrointestinal effects at high doses (Güçlü-Üstündağ&Mazza, 2007). The substantial phenolic content supports the antioxidant potential of C. papaya, relevant for nutritional strategies addressing oxidative stress. The methodological emphasis on reproducibility from controlled drying to validated spectrophotometric assays directly addresses a major limitation in current phytochemical research, enabling different research groups to generate comparable, high-quality data essential for public health decision-making.

4. Conclusion

This study presents a validated, comprehensive protocol for the phytochemical characterization of traditional food plants, specifically applied to Carica papaya leaves and seeds. The methodology ensures reproducibility from plant material handling through quantitative analysis. The successful application revealed distinct phytochemical profiles dominated by saponins and phenolic compounds. By providing this standardized toolkit, we address critical barriers to the evidence-based integration of traditional food plants into public health nutrition strategies, supporting the development of safe, effective, and sustainable nutritional interventions.

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Conflict of Interest

The authors declare no conflict of interest.

Authors' Contributions

H.A.A.: Conceptualization, Methodology, Investigation, Writing – Original Draft. T.J.O.: Writing – Review & Editing, U.I.N, A.I & A.O.O.: Validation, A.B.C & B.J.U.: Formal Analysis. All authors read and approved the final manuscript.

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