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Evaluation of Phytochemical and Antioxidant Properties of *Musa sapientum* (banana) Leaves Extract

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ABSTRACT

In Northwestern Nigeria, *Musa sapientum* (banana leaves) are often underutilized, being regarded primarily as waste or animal feed. This study was therefore conducted to investigate their phytochemical composition and antioxidant properties. The leaves were air-dried, powdered, and extracted using methanol. Standard analytical methods were employed for both qualitative and quantitative assessments. The qualitative phytochemical screening revealed the presence of saponins, glycosides, alkaloids, cardiac glycosides, steroids, saponin glycosides, balsams, and volatile oils in varying concentrations. Cardiac glycosides were identified at high levels, while saponins, glycosides, tannins, saponin glycosides, and alkaloids were present in moderate concentrations. Balsams were identified in trace amounts. The abundant cardiac glycoside demonstrates its potential in the treatment of cardiovascular diseases. Quantitative phytochemical analysis showed flavonoids (2.15 mg/g), tannins (3.8 mg/g), alkaloids (8.5 mg/g), and saponins (3.3 mg/g). The high alkaloid contents signified the ability of the extract to be used in the development of anticancer, antioxidant and antimicrobial drugs. Additionally, the quantitative determination of antioxidant vitamins revealed concentrations of Vitamin A (4.93 mg/dl), Vitamin E (3.8 mg/dl), and Vitamin C (6.2 mg/dl), with Vitamin C being the most abundant. The abundant Vitamin C contents demonstrated the capacity of the extracts to enhance iron absorption and its potential in strengthening of immune system.

Key words: *Musa Sapientum*, Leaves, Phytochemicals, Antioxidants, Methanolic Extract, Quantitative Analysis

Introduction

For millennia, plants have constituted the foundation of advanced traditional medical systems and continue to serve as a valuable source of novel therapeutic agents. While certain purported medicinal properties have later been shown to be inaccurate, the use of plants in therapy is largely supported by empirical evidence accumulated over centuries of human experience (Gurib-Fakim, 2006). Moreover, Herbal medicine, also referred to as botanical medicine or phytotherapy, entails the use of various plant parts such as leaves, roots, and flowers for their therapeutic properties (Niazi and Monib, 2024). Plants play a vital role in supporting human health and well-being by supplying essential nutrients including vitamins, minerals, and dietary fibre that are necessary for maintaining proper physiological function (Azizi *et al.*, 2023). Furthermore, banana (*Musa sapientum*) ranks as the second most important fruit crop in Nigeria. Its year-round availability, affordability, diverse varieties, palatability, and both nutritional and medicinal benefits contribute to its popularity across all social groups (Nyong, 2025). Banana and plantain are among the most significant fruit crops in tropical regions. Beyond serving as a staple food for millions, bananas also represent an important source of income through both domestic markets and international trade (Gopu *et al.*, 2021). The banana is an herbaceous plant that, although widely regarded as a fruit, is botanically classified as a berry. Among the different species cultivated, *Musa acuminata* (Family: Musaceae) is the most common in Nigeria. Across the country, the leaves are identified by various indigenous names: *Akwa leaves* in Igbo, *Ewe ogeed* in Yoruba, *Ganyen kura* in Hausa, *Ebe oghede* in Edo, *Oghede leaves* in Delta, and *Afia efere* in Ibibio (Okoye, 2022). *M. sapientum* is a rich source of essential minerals, including iron, sodium, potassium, copper, magnesium, and calcium, as well as vitamins such as vitamin C, niacin, thiamine, and folic acid. Beyond its high nutritional profile, it also contains a wide range of bioactive compounds with significant health benefits (Choudhury *et al.*, 2023). Additionally, *M. sapientum* leaves exhibit notable pharmacological properties, including anticholinesterase, anti-inflammatory, antioxidant, and antidiabetic activities (Kumar *et al.*, 2021). In India, *M. sapientum* leaves are traditionally preferred as dining plates owing to their rich polyphenol content, which functions as antioxidants and aids digestion by releasing beneficial compounds such as vitamin C and potassium when hot food is served on them (Kora, 2019). *M. sapientum* stems and leaves have also been reported to contain significant amounts of carotene, nicotinic acid, riboflavin, thiamine, and other essential vitamins (Zou *et al.*, 2022). While extensive research has focused on the nutritional and economic importance of banana fruit, the leaves have received comparatively little scientific attention despite their traditional uses. Investigating the phytochemical profile and antioxidant potential of banana leaves is therefore essential to uncover their possible nutritional, medicinal, and industrial applications.



Figure 1: Picture of *M. sapientum* (banana) tree with leaves in its Natural Habitat

Materials and Methods

Collection and preparation of the Methanol Leaves Extract

Fresh banana leaves were obtained from Government Girls College (GGC), Talata Mafara, Zamfara State. The samples were thoroughly washed, air-dried, and pulverised into fine powder for extraction. Ten (10) grams of the powdered material were macerated in 200 cm³ of methanol and allowed to stay in the dark for a day. A Whatman filter paper was used to filter the mixture which was concentrated to dryness by evaporation (Amedu *et al.*, 2024). The dried extract was stored in clean glass bottles until required for analysis.

Qualitative Phytochemical Screening of *M.*

Sapientum Leaves

Test for Anthraquinones

Exactly, 5 ml of the crude methanolic extract were treated with concentrated H₂SO₄ for hydrolysis which was followed by the addition of 1 ml of dilute ammonia. Anthraquinone presence was indicated by the appearance of rose-pink colouration (Balamurugan *et al.*, 2019).

Test for Alkaloids

Exactly, 5 ml of the extract were combined with 2 ml of hydrochloric acid which was followed by the subsequent addition of one ml of Dragendorff's reagent. The alkaloids presence was indicated by the immediate appearance of orange to red precipitate (Velavan, 2015).

Test for Saponins

Into 20 ml of distilled water, 2 g of the extract in a powdered form was boiled on water bath and the mixture was filtered. Also, 5 ml of distilled water was utilised for the dilution of 10

ml of the filtrate and the mixture was strongly shaken, resulting in a steady and tenacious foam. The addition of three drops of olive oil followed by further shaking led to the formation of an emulsion, which confirmed the presence of saponins (Trease & Evans, 1996).

Test for Saponin Glycosides

To 2.5 ml of the extract, 2.5 ml each of Fehling's solutions A and B were added. The development of a bluish-green precipitate was taken as evidence for the presence of saponin glycosides (El-Oleyi *et al.*, 1994).

Test for Balsams

Two drops of chloride solution were added into a mixture of 2 ml of 90% ethanol and 2 ml of the extract. Balsam's presences were confirmed by the development of dark green colouration (El-Oleyi *et al.*, 1994).

Test for Cardiac Glycosides

Glacial acetic acid containing a drop of Ferric chloride solution was mixed with 2 ml of the extract which was gently layered with 1 ml of sulphuric acid in a concentrated form. The appearance of a brown ring at the junction of the two layers signified a positive reaction (Trease & Evans, 1996).

Test for Glycosides

Two (2) ml of glacial acetic acid were added into 5 ml of the extract, and a drop of ferric chloride solution was added to the mixture. Gently, 1 ml of concentrated sulfuric acid was layered along the side of the test tube. The appearance of a distinct brown ring at the interface indicated the occurrence of deoxy sugars in cardenolides. In certain cases, this was accompanied by a violet ring below the brown band, and a gradual green colouration appeared within the layer of acetic acid (Balamurugan *et al.*, 2019).

Test for Tannins

A measured volume of the extract (5 ml) was combined with a few drops of neutral 5% Ferric chloride solution. The immediate appearance of a dark green coloration served as evidence for the presence of tannins (Balamurugan *et al.*, 2019).

Test for Volatile Oils

One ml of the extract was treated with an equal volume of diluted hydrochloric acid. The presence of volatile oils was indicated by the formation of a white precipitate (Evans, 1980).

Test for Flavonoids

A portion of the extract was treated with 5 ml of dilute ammonia solution, followed by the addition of concentrated H₂SO₄. The immediate development of a yellow colouration that gradually faded on standing was taken as evidence of flavonoids (Sofowora, 1993).

Test for Steroids

Into a test tube, 2ml of chloroform was added to the 2ml of the extract and concentrated H₂SO₄ was added carefully produce a distinct lower layer. The emergence of a reddish-brown

colouration at the interface was taken as evidence of a steroid nucleus. (Harborne, 1973).

Quantitative Estimation of Phytochemicals

Determination of Total Phenolics

Folin–Ciocalteu colorimetric assay was used for the determination of phenolic content. One ml of the extract was combined with 9 ml of distilled water, and 1 ml of Folin–Ciocalteu reagent was then introduced. After gentle mixing, the mixture was kept for 5 minutes, followed by the addition of 10 ml of 7% sodium carbonate. The final solution was made up to 25 ml with distilled water. A standard calibration curve was generated using gallic acid solutions (20, 40, 60, 80 & 100 µg/ml). At the temperature of 30°C for 1h and 30 minutes, the mixtures were incubated, and absorbance was taken at 550 nm using a UV–Visible spectrophotometer (757) with distilled water and reagents serving as the blank. Results were expressed as gallic acid equivalents (GAE) per gram of extract (Selvakumar *et al.*, 2019).

Determination of Total Alkaloids Content

Harborne (1973) method was used for the estimation of total alkaloid content of the extract. In this procedure, 5 g of the sample material was treated with 200 ml of 10% acetic acid in ethanol and was allowed to stay covered for a duration of 4 hours. After filtration, the extract was reduced on a water bath to one-fourth of its initial volume. Concentrated NH₄OH was then added gradually to precipitate the alkaloids. The precipitate was collected, washed with dilute ammonium hydroxide, dried, and weighed to obtain the alkaloid yield.

Determination of Total Saponins Content

Harborne (1973) gravimetric double extraction procedure was utilised for the determination of saponin content. Briefly, 5g of the powdered sample was extracted twice with 20% aqueous ethanol under heat (55°C, 90 min) and the pooled extracts were concentrated to ~40 ml. The concentrate was partitioned against diethyl ether until clear, followed by extraction with n-butanol. The combined butanoic layer was washed with 5% NaCl, evaporated to dryness, oven-dried at 60 °C, cooled in a desiccator, and weighed. The outcomes were presented as the initial sample weight percentage.

Determination of Flavonoid Content

For flavonoid determination, 10g of the sample (leaves) were successively extracted with 100 ml of 80% aqueous methanol at ambient temperature. The methanol extract was then filtered through Whatman filter paper, and the filtrate was evaporated to dryness on a water bath. The resulting residue was dried and was repeatedly weighed until a constant weight was achieved by Krishnaiah *et al.* (2009). The difference in weight was taken as the flavonoid content and expressed as a percentage.

Antioxidant Activity Assays

Determination of Ascorbic Acid (Vitamin C)

The spectrophotometric determination of vitamin C was carried out according to Rutkowski *et al.* (1998). One ml of the sample was mixed with 1 ml of prepared reagent (PR) in a centrifuge tube for 30 minutes at room temperature. The mixture was then centrifuged at $7000 \times g$ for 10 minutes, and the supernatant collected for analysis. For the standard, the same procedure was followed, but 1 ml of vitamin C solution was used in place of the test sample and centrifugation was omitted. For the absorbance measurement, UV-Visible Spectrometer (757) was used. The sample absorbance (A_x) and the absorbance of standard (A_s) was taken at 700 nm.

Determination of Vitamin A

The quantification of vitamin A was performed following Rutkowski *et al.* (2006). Into a centrifuge tube, 1ml of potassium hydroxide was mixed with 1ml of the extract and the mixture was shaken for 60 seconds and incubated at 60°C for 20 minutes. After cooling, 1 ml of xylene was added, shaken briefly, and centrifuged at $1500 \times g$ for 10 minutes. The xylene layer was collected, and its absorbance (A_1) taken at 335 nm, using xylene as the blank. The sample was subsequently irradiated with UV light for 30 minutes, after which absorbance (A_2) was recorded from UV-Visible Spectrometer (757).

Determination of Vitamin E Content

Spectrophotometric estimation of vitamin E was carried out using the procedure of Rutkowski *et al.* (2005). Accurately, 0.5 ml aliquot of the sample was mixed with 0.5 ml of anhydrous ethanol in a tightly stoppered centrifuge tube and shaken for 60 seconds. To this mixture, 3 ml of xylene was added, shaken again for 1 minute, and centrifuged at $1500 \times g$ for 10 minutes.

Table 2: Quantitative Phytochemical Compositions of *M. Sapientum* Leaves Extract

Phytochemicals	Amount/Quantity (g%)
Flavonoids	2.15 ± 0.1
Tannins	3.8 ± 0.02
Alkaloid	8.5 ± 0.3
Saponin	3.3 ± 0.1

Values are expressed as mean \pm standard deviation

Table 3: Antioxidant vitamins of *M. Sapientum* Leaves Extract

Vitamins	Concentration (mg/dl)
A	4.93 ± 0.12
E	3.8 ± 0.1
C	6.2 ± 0.3

The results are demonstrated as mean \pm standard deviation.

Statistical Analysis

The statistical analyses were conducted using GraphPad Prism software version 5.01 (GraphPad Software Inc., San Diego, CA, USA). Data were reported as mean \pm standard deviation.

The resulting xylene extract was used for analysis by adding 1.5 ml of the extract to 0.25 ml of bathophenanthroline solution in a clean test tube. After thorough mixing, 0.25 ml of FeCl_3 solution and 0.25 ml of H_3PO_4 solution were added sequentially to prepare the test sample. Standard solutions were prepared in the same way, replacing the test sample with 0.5 ml of α -tocopherol or Trolox standard solution. In the case of the α -tocopherol standard, distilled water was used instead of ethanol, and centrifugation was omitted. Absorbance readings for the sample (A_x) and standard (A_s) were obtained at 539 nm using UV-Visible Spectrometer (757), with a reagent blank prepared by replacing the extract with distilled water.

Results

Table 1: Qualitative Phytochemical Analysis of *M. Sapientum* Leaves Extract

Phytochemical Constituent	<i>M. Sapientum</i> Leaves Extract
Flavonoids	-
Tannins	-
Saponins	++
Glycosides	++
Alkaloids	++
Cardiac Glycosides	+++
Steroids	++
Saponin Glycosides	++
Balsams	+
Anthraquinones	-
Volatile Oils	+

- = not detected, + = present, ++ = moderately abundant, +++ = highly abundant

Discussion

The qualitative phytochemical screening indicated the presence of alkaloids, glycosides, cardiac glycosides, steroids, saponins, saponin glycosides, balsams, and volatile oils in varying concentrations (Table 1). Cardiac Glycosides were present at high concentration while Saponins, Glycosides, Tannins, Saponin Glycosides and Alkaloids are present at medium concentration. Conversely, Balsams were found also present but in minute amount. The qualitative phytochemical screenings in this study revealed higher levels of phytoconstituents compared to those reported by Bisht *et al.* (2016). Conversely, the qualitative phytochemicals reported in this study are similar to those reported by Ogu *et al.* (2024).

The quantitative phytochemical analysis presented in Table 2 showed flavonoids (2.15), tannins (3.8), alkaloids (8.5), and saponins (3.3). Among these, alkaloids were the most abundant, followed by tannins, saponins, and flavonoids. These findings support the traditional medicinal use of banana leaves in managing various ailments, including the prevention of malignant ulcer growth, as well as the treatment of diarrhoea and dysentery (Rajesh, 2017). Phytochemical constituents are recognized for imparting a wide range of therapeutic benefits to plants. Among them, indole-containing alkaloids have been

documented to exhibit potent pharmacological activities, including anti-HIV, anticancer, and antioxidant effects (Rajput *et al.*, 2022). Tannins have been utilized for their pharmacological roles, particularly as anti-hemorrhoidal and antidiarrheal agents, as well as in the treatment of haemostatic disorders (Sharma *et al.*, 2019). Saponins are well recognized as bioactive compounds with multiple pharmacological properties, including antitumor, anti-inflammatory, hypoglycaemic, antioxidant, and hypocholesterolaemia activities (Sharma *et al.*, 2023). Various classes of flavonoids have been isolated, and they exhibit a wide range of biological activities, including anticancer, antibacterial, antifungal, antidiabetic, antimalarial, neuroprotective, cardioprotective, and anti-inflammatory effects (Ullah *et al.*, 2020).

The quantitative analysis of antioxidant vitamins (Table 3) showed concentrations of Vitamin A (4.93 mg/dl), Vitamin E (3.8 mg/dl), and Vitamin C (6.2 mg/dl). Vitamin C was present in the highest concentration, followed by Vitamin A, while Vitamin E had the lowest concentration. Vitamin C exhibits strong antioxidant potential by protecting cellular structures against the damaging effects of free radicals. Additionally, it plays a crucial role in enhancing iron absorption by converting dietary iron into a more readily absorbable form in the intestines (Sinbad *et al.*, 2019). Vitamin A is vital for numerous physiological processes, including vision, reproduction, immune function, cell differentiation, proliferation, apoptosis, maintenance of epithelial tissues, brain function, and embryonic growth and development, among others (Meléndez-Martínez, 2019). Retinoic acid plays an essential role in regulating the differentiation, maturation, and function of innate immune cells. Vitamins C and E, as natural antioxidants, mitigate oxidative stress, and it has been postulated that these vitamins may slow the progression of AIDS (Huang *et al.*, 2018).

Dietary vitamin E supplementation has been shown to improve both cell-mediated and humoral immune functions in a range of animal species (Lee & Han, 2018).

Limitations of the Study

Regardless of the significant insights highlighted by this research, certain limitations should be acknowledged. First, the study utilized only one solvent for phytochemical extraction. The polarity of the solvent plays a critical role in determining the efficiency of extraction of various phytochemical classes. Thus, the utilization of a one solvent may have limited the recovery of some compounds, especially the ones with markedly different polarity profiles.

Also, the research centered exclusively on quantitative determination of selected phytochemicals without further chromatographic characterization. Advanced analytical techniques such as Gas Chromatography-Mass Spectrometry (GC-MS), Liquid Chromatography-Mass Spectrometry (LC-MS) and High Performance-Liquid Chromatography (HPLC) could give succinct information on the explicit bioactive compounds present in *M. sapientum* leaves.

Conclusion

Based on the findings of this research, *M. sapientum* (banana) leaves are rich in bioactive compounds such as cardiac glycosides, alkaloids, tannins, saponins among others and antioxidant vitamins namely Vitamins C, A and E, underscoring their potential as valuable dietary supplements. Furthermore, their unique phytochemical profile positions them as a promising resource for the development of innovative nutraceuticals and pharmaceutical agents/therapeutics.

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