

Original Article

Proximate Composition, Mineral Profile, Phytochemical Constituents, and Antioxidant Potential of the Methanol Extract and Solvent Fractions of *Hillieria Latifolia* (whole plant)

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Abstract - Research was carried out on the proximate, mineral, and phytochemical investigations, together with the antioxidant potential of the methanol extract and solvent fractions of *Hillieria latifolia* (whole plant) (Phytolaccaceae). The plant was collected from Ikire, Osun State, Nigeria, authenticated, and extracted using absolute methanol by Soxhlet extraction. The crude methanol extract was subjected to solvent partitioning to obtain aqueous and chloroform fractions. Phytochemical examination showed that coumarins, saponins, terpenoids, reducing sugars, phenolic constituents, and cardiac glycosides were present, whereas phlobatannins were not detected. Proximate examination showed moisture content (10.30%), protein (12.02%), fat (9.50%), fiber (14.89%), ash (20.20%), and carbohydrates (33.09%). Mineral content revealed Calcium (2885.76 ppm), Potassium (2698.30 ppm), Magnesium (1458.72 ppm), Iron (69.50 ppm), Sodium (48.76 ppm), Zinc (6.60 ppm), Copper (1.10 ppm), and Manganese (21.42), while the Lead and Cadmium contents were negligible. The aqueous fraction had the highest total phenolics (540.61 ± 8.98 mg GAE/g) and flavonoids (416.13 ± 8.93 mg QE/g), which correlated with its superior antioxidant activity as demonstrated by its lowest DPPH IC₅₀ (79.75 µg/mL) and highest FRAP value (853.05 mg AAE/g). These findings suggest that *H. latifolia* has a favourable nutritional profile, rich mineral content, and potent antioxidant properties supporting its nutritional and medicinal potential.

Keywords - Phytochemical Analysis, Antioxidant Activities, Mineral Content, Medicinal Plants.

1. Introduction

Native plants are recently being recognized as vital food resources for healthy communities. They provide affordable sources of protein, energy, vitamins, minerals, and essential amino acids (FAO, 2023). Malnutrition can be considerably decreased by consuming these plant-based foods, especially in rural regions. Many plants have phytochemicals that have medicinal properties in addition to proximate nutrients (Sofowora, 2008). Phenolic components of these phytochemicals, such as flavonoids, dietary glutathione, and tannins, are widely known for their excellent antioxidant properties, which may help prevent conditions including diabetes, arthritis, cancer, and asthma (Shahidi and Ambigaipalan, 2015; Shi et al., 2022). Plant-derived antioxidants protect the body systems through various mechanisms, such as scavenging Reactive Oxygen Species (ROS), decomposing peroxides, inhibiting oxidative enzymes, and acting synergistically with other antioxidant compounds. (Awah et al., 2012; Gülçin, 2020). The potential

adverse health effects of certain synthetic antioxidants have led to the need for safer, more sustainable plant-derived alternatives (Subedi et al., 2014).

Hillieria latifolia (*H. latifolia*), found within the Phytolaccaceae family, is known in English as forest spinach or pepper herb. Across West Africa, *H. latifolia* is known by several indigenous names, showing its use and recognition among different local communities. It is recognized as “Anafranaku” by the Asantes in Ghana, “Aka ato” in the Igbo language of Southeast Nigeria, “Ogo” in the Yoruba language of Southwest Nigeria, and “Efehentok” among the Ibibio people in the South-South region of Nigeria (Johnson et al., 2017; Ajayi et al., 2024). This plant is found in various regions of Africa, including Nigeria, Angola, Cameroon, Madagascar, and Ghana (Royal Botanic Gardens, Kew, 2026). In Nigeria, the species can be found in several regions, including Ondo and Oyo States (Ajayi et al., 2024). The plant is consumed as a leafy vegetable in Ghana,



Nigeria, and Cameroon. It is also used in ethnomedicine to treat fever, skin diseases, feverish conditions, rheumatism, urethral discharges, and other health conditions in different parts of Africa (Ajayi et al., 2024; Johnson et al., 2017).

Previous researchers have shown that *H. latifolia* contains different phytochemicals, including alkaloids, anthraquinones, triterpenoids, tannins, flavonoids, and cardiac glycosides in various parts of the plant (Woode *et al.*, 2011; Johnson *et al.*, 2017; Johnson *et al.*, 2018). These have been attributed to the reported diverse therapeutic efficacies, including anti-inflammatory, antipyretic, anti-arthritis, wound-healing, antioxidant, and antimicrobial activities, as well as the plant's *in vitro* anti-plasmodial activity (Abotsi *et al.*, 2011; 2012; Dapaah *et al.*, 2017; Johnson *et al.*, 2017). Although the biological effects of *H. latifolia* have been examined, information on its proximate composition, mineral profile, and radical-scavenging potential of the whole plant remains limited. To address these limitations, this study aims to evaluate the proximate and mineral composition of whole-plant *H. latifolia*; quantify the phenolic and flavonoid contents; and determine the antioxidant efficacies of the crude methanol extract and its aqueous and chloroform fractions.



Fig. 1 *H. Latifolia* collected from Ikire Community (Osun State, Nigeria)

2. Methodology

2.1. Chemicals and Reagents

Petroleum ether, ethanol, chloroform, hydrochloric acid (HCl), sulphuric acid (H₂SO₄), aluminium chloride (AlCl₃), glacial acetic acid, sodium carbonate (Na₂CO₃), sodium hydroxide pellets (NaOH), ascorbic acid, ferric chloride (FeCl₃). Fehling's solutions A and B, Folin-Ciocalteu reagent, gallic acid, potassium acetate, quercetin, 2,2-diphenyl-1-picrylhydrazyl (DPPH, > 95%), methanol, 2,4,6-tripyridyl-s-triazine (TPTZ), ferric chloride hexahydrate (FeCl₃·6H₂O), acetate buffer. All chemicals and reagents employed in this research were of analytical quality (≥99%)

and were purchased from Sigma-Aldrich (USA) and BDH Chemicals (UK).

2.2. Plant Collection and Identification

The whole plant of *H. latifolia* used for the experiment was collected from a cocoa farm in Agbegi village, located in Ikire, Osun State. Botanical identification was done in June 2021 by Dr. Samuel Odewo at the Forest Research Institute of Nigeria (FRIN), Ibadan, Oyo State, Nigeria. Voucher specimen no. FHL113269 has been deposited at the FRIN Herbarium.

2.3. Sample Preparation

The harvested plant was cut into pieces and rinsed twice with deionized water. After three weeks of air drying in the laboratory, the plant was further dried at 50 °C in an electric oven. The dried material from the plants was then crushed into a coarse powder using an electric mill (by Chris Norris, England) and later stored in an airtight container for analysis.

2.4. Extraction and Fractionation

Approximately 1.0 kg of powdered *H. latifolia* was subjected to extraction using a 2 L Soxhlet extraction apparatus with approximately 10 L of 100% methanol. Extraction was conducted for approximately 24 hours, yielding 114.95 g (11.50%). The Methanol Extract (ME) was concentrated under low pressure using a rotary evaporator at approximately 40 °C. The yield (%) of the extract was calculated using equation 1 below.

$$\text{Yield (\%)} = \frac{a}{b} \times 100 \quad (1)$$

where:

a = weight of the dried crude extract (g)

b = dry weight of the powdered plant material (g)

Approximately 50 g of the crude methanol extract was re-dissolved in water and partitioned repeatedly using chloroform (200 mL × 4) in a 1000 mL separating funnel. Each fraction was concentrated separately to obtain an aqueous fraction (26.32 g) and a chloroform fraction (15.04 g). The crude extract and the fractions were refrigerated at 4 °C until needed (Ikpefan *et al.*, 2020).

2.5. Preliminary Phytochemical Screening

The crude extract of *H. latifolia* was subjected to phytochemical evaluation using standard procedures to detect secondary metabolites, including saponins, terpenoids, coumarins, reducing sugars, phenols, cardiac glycosides, and phlobatannins (Sofowora, 2008; Trease and Evans, 2009).

2.6. Proximate Analysis

The plant's proximate analysis, comprising moisture (M), ash (A), crude fiber (F), crude protein (P), crude fat (FA), and carbohydrate, was conducted based on the AOAC (2023) protocols. The carbohydrate value was determined via

the difference method, as depicted in Equation 2. The test was repeated thrice ($n = 3$), and the mean was recorded.

$$\begin{aligned} \text{Carbohydrate (\%)} &= 100 \\ &- [M (\%) + P (\%) + FA (\%) + F (\%) \\ &+ A (\%)] \end{aligned} \quad (2)$$

2.7. Mineral Analysis

The mineral analysis of the sample (1 g) was performed by dry ashing in a muffle furnace at 550 °C for 5 hrs, followed by acid extraction as outlined by Anuforo *et al.* (2017). The resulting filtrate was kept for mineral composition analysis. Calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), lead (Pb), and cadmium (Cd) were determined using an atomic absorption Spectrophotometer (AAS, Buck Scientific VGP 210, USA), in contrast, sodium (Na) and potassium (K) were determined using a flame photometer (Sherwood Model 410, UK) as per AOAC methods (AOAC 2023). Results were expressed as parts per million (ppm) of dry weight.

2.8. Total Phenolic Content (TPC)

The phenolic contents were determined using the Folin–Ciocalteu method as described by Gbolade *et al.* (2020). Each of 0.10 g of gallic acid (reference standard), the crude extract, and the fractions was dissolved in 100 mL of water to obtain a 1 mg/mL stock solution. Different concentrations of gallic acid ranging from 0.02 to 1.00 mg/mL were prepared. Each sample (1 mL) was incubated with 2 mL of Folin–Ciocalteu reagent and 2 mL of 7.5% sodium carbonate for 45 minutes in a water-bath heater at 40 °C. The absorbance of the resulting mixture was measured at 765 nm using a UV-Visible spectrophotometer (Model SM23A; Microfield®, England). The experiment was conducted in triplicate for the crude extract and the fractions. A blank was prepared by combining all reagents except the extract/standard. The TPC was calculated using a gallic acid calibration curve, and the results were expressed as milligrams of gallic acid equivalents (GAE) per gram of dry samples. The TPC values of the plant samples were estimated using equation 3

$$\text{TPC (mg GAE/g)} = C \times \frac{V}{m} \quad (3)$$

where:

C = concentration of sample obtained from the gallic acid calibration curve (mg/mL)

V = final volume of extract (mL)

m = mass of each plant sample (g)

2.9. Total Flavonoid Content (TFC)

The estimation of flavonoids was done by modifying the colorimetric method using aluminum chloride by Shraim *et al.* (2021). 0.10 grams of quercetin (as reference standard), crude extract, and the fractions were individually weighed

and dissolved in 100 ml of water to form a stock solution of 1 mg/ml. A 1.0 ml sample of the extract (1 mg/ml) was mixed with 0.1 ml of 10% aluminum chloride solution, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water.

The mixture was then kept for 40 minutes at room temperature, and the absorbance was taken at 415 nm using a UV-Visible Spectrophotometer (Model SM23A; Microfield®, England). The experiments were done in triplicate for both the crude extract and the fractions. Quercetin standard solutions were prepared at concentrations (0.02 to 1.00 mg/mL), and their respective absorbances were plotted against their concentrations. The TFC in the extracts was estimated using the standard curve of quercetin. TFC of samples was reported in terms of mg of quercetin equivalents per gram of dried sample (mg QE/g). The TFC of the crude extract and solvent fractions was determined using equation 4:

$$\text{TFC (mgQE/g)} = C \times \frac{V}{m} \quad (4)$$

where C is the concentration of the sample, extrapolated from the quercetin calibration curve (mg/mL); V is the final volume of the plant sample (mL); and m is the mass of the sample used (g).

2.10. DPPH Antioxidant Activity

The radical-scavenging activity of the crude extract and its fractions towards the DPPH radical was evaluated using a UV Spectrophotometer (SM23A model; Microfield®, England) at a wavelength of 517 nm as per the method described by Gbolade *et al.* (2020). Solutions of the crude extract, solvent fractions, and ascorbic acid (standard) were prepared in methanol at concentrations ranging from 50 to 300 µg/mL. 1 mL of each sample was taken in a properly labeled test tube. Then, 2 mL of 0.1 mM DPPH in methanol was added. Negative control was prepared by adding 2 mL of DPPH solution to 1 mL of methanol. The mixture was kept for 30 min, and the absorbance was read at 517 nm. The antioxidant activity was calculated using equation 5:

$$\% \text{ inhibition} = \left\{ \frac{A_2 - A_1}{A_2} \right\} \times 100 \quad (5)$$

Where A2 is the absorbance of the control, and A1 is the absorbance of the sample.

The IC₅₀ values (µg/mL) were calculated using linear regression.

2.11. Ferric-Reducing Antioxidant Potential (FRAP) Assay

The FRAP assay involves the reduction of the Fe³⁺–TPTZ complex to the Fe²⁺–TPTZ complex by electron donation from antioxidants. A modified method, as described by Xiao *et al.* (2020), was employed for this assay.

A 300 mM acetate buffer (pH 3.6), 10 mM TPTZ, and 20 mM FeCl₃·6H₂O were prepared separately. The three

solutions were prepared at a 10:1:1 (v/v) ratio of acetate buffer:TPTZ:FeCl₃·6H₂O to yield the working FRAP reagent. The resulting FRAP reagent was warmed to about 37 °C for 10–15 minutes before use. Each sample (100 µL; 1.0 mg/mL) was mixed with 2.9 mL of the FRAP mixture and incubated at room temperature for 30 minutes. Absorbance was taken at 593 nm. The experiment was conducted in triplicate for the crude extract and the fractions. The calibration curve was plotted using standard solutions of ascorbic acid (0.2–1.0 mg/mL). Results were reported as milligrams of ascorbic acid equivalent per gram of plant sample (mg AAE/g).

2.12. Statistical Analysis

Microsoft Excel was employed to analyze the data, which were expressed in terms of mean ± standard error of the mean (SEM). Statistical analysis of differences was carried out through one-way Analysis of Variance (ANOVA) and Tukey's multiple comparison post hoc test. $p < 0.05$ was accepted as significant.

3. Results and Discussion

3.1. Extract Yield and Phytochemical Evaluation

Extraction of 1.0 kg of powdered *H. latifolia* whole plant using methanol in a Soxhlet apparatus yielded 114.95 g (11.50%) of Methanol Extract (ME). Subsequent solvent partitioning of 50.0 g of the crude methanol extract yielded 26.32 g (52.64%) of the Aqueous Fraction (AF) and 15.04 g (30.08%) of the chloroform fraction (CF). The higher yield obtained from the aqueous fraction suggests that the crude extract of *H. latifolia* contains predominantly polar constituents. Because of its effectiveness in removing bioactive compounds from dried plant materials, the Soxhlet process was used. Soxhlet extraction provides a quicker and more practical way of comprehensive extraction as compared to other techniques like decoction, percolation, and maceration. However, the disadvantages of this method include its cost and incompatibility in extracting thermolabile constituents, which may undergo thermal degradation under prolonged heating (Abubakar and Haque, 2020).

Table 1. Results of the phytochemical screening of *H. latifolia* (whole plant)

Class of phytochemical	Results
Saponins	+
Terpenoids	+
Coumarins	+
Reducing sugar	+
Cardiac glycosides	+
Phenolic compounds	+
Phlobatannin	-

+ present - absent

Phytochemicals form the foundation of medicinal plants' biology and pharmacology. They also give the colour, taste, and smell to the plants. (Mazid *et al.*, 2011). Phytochemical screening of the methanol extract of *H. latifolia* showed that

saponins, terpenoids, coumarins, reducing sugars, cardiac glycosides, and phenols were present (Table 1). These results tally with the findings of Johnson *et al.* (2017), Abotsi *et al.* (2011), and Amponsah *et al.* (2014).

The anti-inflammatory and hypocholesterolemic actions of saponins are well-known (Wijesekara *et al.*, 2024; Jiang *et al.*, 2025). The decrease in cholesterol concentration in the bloodstream takes place through many distinct mechanisms related to absorption and metabolism (Jiang *et al.*, 2025). Saponins are capable of showing gastroprotective effects and help in the prevention of gastric ulcers (Yadav *et al.*, 2024). Terpenoids represent one of the most diverse kinds of phytochemicals with high pharmaceutical importance. As is well-known, the terpenoids have a broad spectrum of biological actions, which include anti-inflammatory, antioxidant, and anticancer actions (Yang *et al.*, 2020; Câmara *et al.*, 2024). Coumarins represent another kind of secondary plant metabolites, which possess many pharmacological actions, including anti-inflammatory, anticoagulant, antimalarial, and hepatoprotective actions (Venugopala *et al.*, 2013).

The main functions of reducing sugars are to act as easily available energy sources and participate in the metabolic processes of plants (Rolland *et al.*, 2006). Reducing sugars also act as building blocks in the synthesis of glycosides and other biologically active substances (Bar-Peled and O'Neill, 2011). Cardiac glycosides have found use in medicine as treatment agents in heart failure and certain forms of arrhythmia. Apart from being used as cardiac medications, some of the cardiac glycosides have also been found useful in their anti-cancer activity. The detection of phenolic constituents in the extract provides further evidence of the potential antioxidant activity of *H. latifolia*. Phenolic compounds are well known for their ability to act as scavengers of free radicals and are involved in a number of biological activities, such as antioxidant, anti-inflammatory, anti-ulcer, and anticancer (Shahidi and Ambigaipalan, 2015; Panche *et al.*, 2016). Hence, the identification of these compounds is also another piece of evidence for the possible medical utility of *H. latifolia*, especially in treating or preventing oxidative stress-related and other chronic conditions.

3.2. Proximate Analysis

Table 2. Proximate analysis of *H. latifolia*

Parameters	Mean (%) ± SEM
Moisture content	10.30 ± 0.62
Ash content	20.20 ± 0.87
Crude fat	9.50 ± 0.72
Crude fibre	14.89 ± 0.96
Crude protein	12.02 ± 0.48
Carbohydrate	33.09 ± 0.49

The values are presented as mean ± SEM (n=3)

Proximate composition still forms a basic approach used in determining the nutritional value of plant-based foods (AOAC, 2023). Proximate analysis of *H. latifolia* conducted in this experiment showed that the plant is an excellent source of important nutrients (Table 2).

The moisture content of *H. latifolia* was $10.30 \pm 0.62\%$ w/w. This value was lower than the 36.44% and 22.24% reported by Gbadamosi *et al.* (2024) for *Chromolaena odorata* and *Ipomoea involucre*, respectively. Moisture content is a crucial quality parameter since it affects stability, shelf life, and the likelihood of contamination of plant material with microorganisms (Adeogun *et al.*, 2018; Bennett *et al.*, 2024). Overall, moisture content increases the instability of crude drugs. On the other hand, high moisture content promotes the development of microorganisms and degradation of the plant material (Adeogun *et al.*, 2018; Bennett *et al.*, 2024). A value below 12% is usually recommended as an appropriate moisture content for storing dried plant materials (WHO, 2018). Thus, the moisture content obtained in the current study is acceptable, which means that the powdered plant material of *H. latifolia* probably has good storage stability and a reduced likelihood of microbiological deterioration or degradation of the bioactive components. Ash content gives an estimation of the amount of inorganic minerals in plant material. Minerals contained in plants perform many physiological functions, such as maintaining acid-base balance, electrolyte balance, enzyme activity, and metabolic processes (e.g., glucose metabolism) (Vunchi *et al.*, 2011). Relatively high ash content indicates considerable mineral content and contributes to the nutritional properties of edible plants (Nielsen, 2017). Therefore, the relatively high ash content reported in this study implies that *H. latifolia* is a good source of dietary minerals and may prevent micronutrient deficiencies.

The percentage of crude fat was $9.50 \pm 0.72\%$, which indicated a relatively high lipid content in the plant material. Lipids provide an energy source for physiological activities and influence body temperature regulation and other processes, such as organ protection (Gropper *et al.*, 2022). The percentage of crude fibre ($14.89 \pm 0.96\%$) was similar to 14.67% found for *Jatropha tanjorensis* (Bennett *et al.*, 2024). This relatively high fibre percentage indicates that *H. latifolia* can be used as an alternative source of dietary fibre. Intake of dietary fibres in sufficient portions is related to reduced incidences of several health problems, such as cardiovascular conditions, diabetes, hypertension, obesity, and digestive problems (Waddell and Orfila, 2023). The percentage of crude protein was $12.02 \pm 0.48\%$, indicating a moderately high percentage of protein in *H. latifolia*. The value of the percentage of protein was higher than the 6.20% and 1.30% found for *Jatropha tanjorensis* and *Bryophyllum pinnatum*, respectively, by Bennett *et al.* (2024). Proteins are essential for biological functions such as enzymes,

hormones, plasma protein synthesis, immunological activities, and tissue repair and growth (Gropper *et al.*, 2022). Carbohydrates made up the highest proximate component at $33.09 \pm 0.49\%$. This relatively high carbohydrate content indicates that *H. latifolia* can be used as a potential dietary energy source. However, the value was higher than the 15.00% and 25.37% found for *Chromolaena odorata* and *Ipomoea involucre*, respectively, by Gbadamosi *et al.* (2024). Carbohydrates, which are mainly produced by photosynthesis in plants, act as the main energy source in humans and animals.

Proximate composition analysis conducted in the current study greatly varies from that carried out by Ogundare *et al.* (2023) on the whole plant of *H. latifolia*, which gave moisture content of 20.30%, ash content of 5.22%, lipid content of 2.05%, crude fibre content of 22.06%, protein content of 3.88%, and carbohydrate content of 54.26%. Some of the factors that might account for the disparity between results obtained in the current study and those published earlier include the plant part studied, geographical location, soil composition, environmental conditions, maturity of the plant, among others.

3.3. Elemental Analysis

Table 3. Analysis of some mineral compositions of *H. latifolia* (whole plant)

Mineral Compositions	Concentration (ppm)
Calcium	2885.76 ± 11.01
Magnesium	1458.72 ± 5.56
Potassium	2698.30 ± 9.23
Sodium	48.76 ± 2.73
Iron	69.50 ± 4.45
Zinc	6.62 ± 0.74
Copper	1.19 ± 0.16
Manganese	21.42 ± 0.92
Lead	0.01 ± 0.00
Cadmium	0.00 ± 0.00

The values above are mean $\pm$ SEM (n=3).

The mineral content analysis showed high levels of various essential minerals like macro minerals and micro minerals, whose concentrations were determined in parts per million. The minerals identified included Calcium (Ca), whose concentration was highest, with an average of 2885.76 ± 11.01 ppm; followed by Potassium (K) with 2698.30 ± 9.23 ppm; Magnesium (Mg) 1458.72 ± 5.56 ppm; Iron 69.50 ± 4.45 ppm, and Cadmium not being identified (Table 3).

The mineral composition of *H. latifolia* showed that it contained substantial amounts of some macronutrients and micronutrients, which might make it useful for nutrition. The calcium content was found to be significant and necessary

for healthy bone and teeth formation and also for proper muscle contraction, blood coagulation, and enzymatic activity (Deluque et al., 2025). Calcium deficiency can lead to bone mineral density disorders such as osteopenia and also influence cardiovascular system functioning (Razzaque, 2025). The amount of magnesium in *H. latifolia* was equal to 1458.72 ppm, which was higher than that of *Monodora myristica* and *Allium cepa*, but lower than that of *Xylopia aethiopica*, according to Adebola et al. (2017). Magnesium plays a role in many physiological functions, such as metabolism, neuromuscular activity, and bone formation (Gröber et al., 2015). While magnesium is important for proper physiological activity, its excess, especially when taken as supplements, can lead to side effects like nausea and diarrhea (Goldhaber, 2003).

Potassium is critical for effective nerve transmission, fluid balance, and blood pressure management (Toft et al., 2024). Potassium may cause vasodilation, resulting in dilation of blood vessels and enhancement of blood flow (Adeogun et al., 2018). Potassium intake should be at 3.5 grams per day based on WHO recommendations (Reddin et al., 2023). The sodium concentration was low at 48.76 ppm, resulting in a healthy Na: K ratio of 0.018. It is usually preferable to have a low Na ratio since high sodium content compared to potassium causes hypertension and cardiovascular disease (Hisamatsu et al., 2024). The sodium level of this study was low compared to the levels of *Ocimum gratissimum* and *Zingiber officinale* in the study by Adebola et al. (2017). Excessive intake of dietary sodium results in high blood pressure and heart failure (Weaver, 2013).

Iron level was 69.50 ± 4.45 ppm. Iron is a trace element that is mainly found in hemoglobin and a variety of enzymes responsible for oxygen transfer and energy metabolism (Adeogun et al., 2018). Even though iron is an important component that is required for the proper functioning of the organism, its excess can lead to oxidative stress and toxicity, which can cause damage to different organs, among which is the liver (Saleh et al., 2023). The zinc level was 6.62 ppm, which is considerably lower compared to 21.3 ppm found in *Cassia nigricans* (Ayo et al., 2013).

The copper content in this plant was 1.19 ppm, which was far lower than the 41.98 ppm that was found for *Urtica dioica* as described by Kumar et al. (2021). Copper is one of the essential cofactors of some of the enzymes needed for oxidation and reduction reactions (Gropper et al., 2022). Excess accumulation of copper may cause damage to the liver and may lead to copper-related problems like Wilson's disease, especially in people who have a susceptibility to these diseases (Kumar and Roy, 2025). The manganese concentration was 21.42 ppm, which was equivalent to the approximate 20 ppm in *Allium sativa* reported by Adegbola et al. (2017). Manganese is a trace metal needed for some

body functions such as energy production, glucose regulation, immune response, skeletal growth, reproduction, and antioxidants (Li and Feng, 2018). Deficiency of manganese may affect normal bone growth, while its excess will cause neurotoxicity (O'Neal and Zheng, 2015).

H. latifolia has a mineral profile that includes nutritionally relevant components like Mg, K, Ca, Fe, Zn, Cu, and Mn. Low amounts of sodium, together with the beneficial Na content ratio, are among the indicators of the nutritional quality of *H. latifolia*. It should be noted, however, that the nutritional quality of minerals in *H. latifolia* depends not only on their presence but also on their availability and consumption.

However, the amounts of lead and cadmium reported at 0.01 ppm and 0.00 ppm, respectively, were both below the maximum permissible level established by FAO/WHO, hence confirming the safety of *H. latifolia* as a dietary food product (Sulaiman et al., 2024). These toxic heavy metals offer no physiological significance to the body, and thus their chronic effects include anaemia, cancer, neurological disorders, and kidney damage (Lawal and Odukoya, 2021).

3.4. Phenolics and Flavonoid Contents

Table 4. Phenolics and Flavonoid contents of *H. latifolia* (crude and fractions)

Sample	TPC (mg/g GAE)	TFC (mg/g QE)
Aqueous fraction (AF)	540.61 ± 8.98^a	416.13 ± 8.93^a
Chloroform fraction (CF)	420.87 ± 5.23^b	368.40 ± 9.83^b
Methanol extract (ME)	331.93 ± 2.55^c	321.03 ± 9.87^c

The values above are expressed as mean $\pm$ SEM (n = 3). Means with different superscript letters within each column differ significantly ($p < 0.05$) according to one-way ANOVA followed by Tukey's post hoc test.

The highest amount of TPC (540.61 ± 8.98 mg/g GAE) and TFC (416.13 ± 8.93 mg/g QE) were recorded in the AF. This is followed by CF (420.87 ± 5.23 mg/g GAE; 368.40 ± 9.83 mg/g QE), whereas ME showed the lowest values of the two parameters studied ($p < 0.05$) (Table 4). The results demonstrate that the polarity of the solvents used has a notable influence on the yield of the phenolics and flavonoids extracted from the plant material. This can be explained by the high presence of hydroxyl groups in the phytochemicals under study, which form hydrogen bonds easily with polar solvents (Aktas and Kurek, 2024; Shahidi and Ambigaipalan, 2015).

It is worth noting that this finding agrees with other studies, which indicate better phenolic extraction in the

aqueous fraction as opposed to the other solvent fractions (Nasraoui et al. 2026; Ajayi et al., 2024). Although the chloroform fraction (CF) is relatively less polar, it was able to yield moderate amounts of both phenolics and flavonoids, showing the presence of moderately polar phenolic substances. Solvent partitioning may have contributed to the increased presence of these phytochemicals in the AF (Sultana et al., 2009). In addition, the TPC values (332–541 mg GAE/g) recorded in this study were similar to those of Ajayi et al. (2024) for *H. latifolia* but higher than 29.40 mg TAE/g reported by Abotsi et al. (2011). Likewise, the TFC values (321–416 mg QE/g) were higher than those by Ajayi et al. (2024), which indicates that these results are in agreement with previous findings where it was noted that phenolics are found in greater quantities in the aqueous fraction than in the other fractions of solvents used (Nasraoui et al. 2026; Ajayi et al., 2024). In spite of its lesser polarity, the chloroform fraction (CF) was still able to contain high levels of TPC and TFC, showing the presence of moderate phenolic substances. It could be speculated that solvent partitioning has helped in increasing the quantity of such phytochemicals in the AF fraction (Sultana et al., 2009). Besides, the TPC found in this study (332–541 mg GAE/g) was similar to that of Ajayi et al. (2024) for *H. latifolia*, but

higher than 29.40 mg TAE/g reported by Abotsi et al. (2011). The same was true for TFC values (321–416 mg QE/g).

The utilization of Plant materials that have high levels of phenolic compounds is receiving much recognition in the food industry since these materials can possibly lower the oxidation of lipids, hence improve the nutritional value, flavour, and shelf life of food products. It is due to the hydroxyl groups of the phenolic compounds that increase their ability to act as free radical scavengers (Shahidi and Ambigaipalan, 2015; Guan *et al.*, 2021). In Plants, phenolic compounds are categorized into different classes, with flavonoids forming the main class and also the one with bioactivity, possessing antioxidant properties (Panche *et al.*, 2016). The bioactivity of flavonoids is determined by the presence and positioning of hydroxyl (-OH) groups attached to their aromatic ring, affecting their redox and radical scavenging abilities (Panche *et al.*, 2016).

3.5. DPPH Radical-Scavenging Activity (Antioxidant Property)

The scavenging capacity of the ascorbic acid (standard), crude extract, and fractions of *H. latifolia* has been presented in Figure 2. The IC₅₀ of the extract, fractions, as well as ascorbic acid, has been presented in Figure 3.

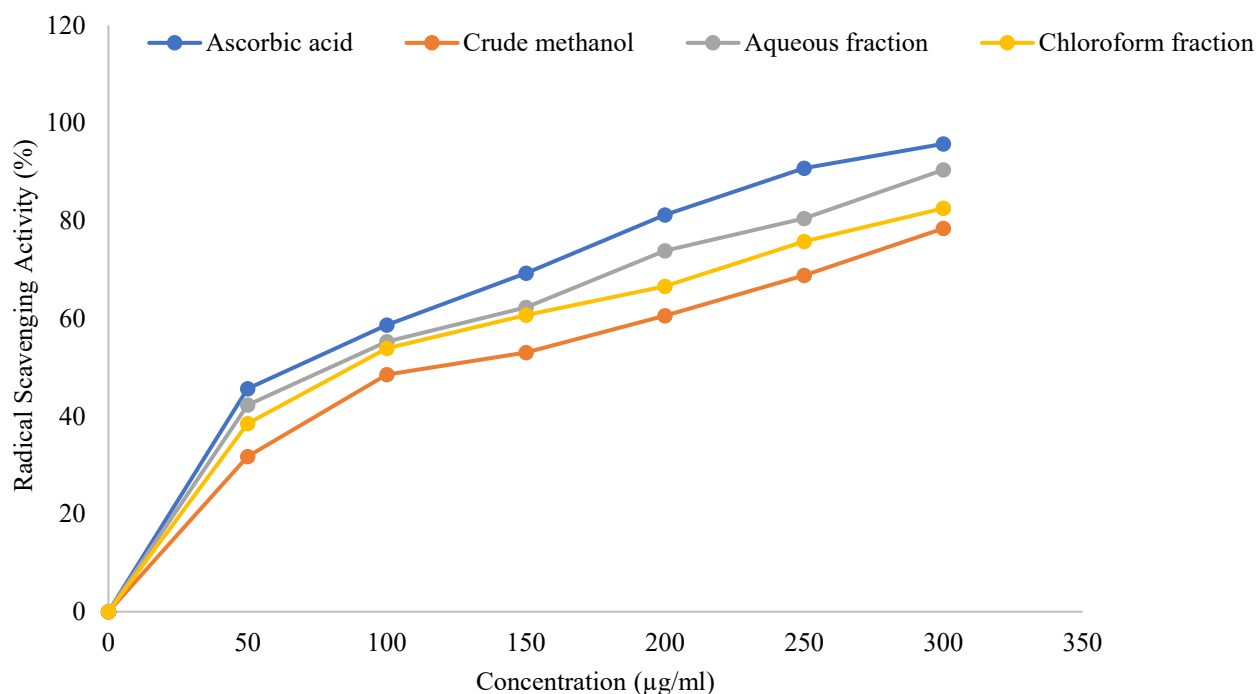


Fig. 2 DPPH antioxidant activity of *H. latifolia* extracts compared with ascorbic acid

H. latifolia was investigated for antioxidant potency using the DPPH assay. The results indicated that the antioxidant activity is concentration-dependent in all the samples as well as in the control (ascorbic acid). This shows that the studied plant extracts possess hydrogen- or electron-

donating activity, which helps them to neutralize free radicals (Panche *et al.*, 2016).

Ascorbic acid had the highest scavenging activity (95.72%) at 300 µg/mL, followed by the aqueous fraction

(90.40%), the chloroform fraction (82.53%), and the methanol extract (78.41%) (Figure 2). At all concentrations, one-way ANOVA showed significant differences ($p < 0.05$) among the extracts. The higher antioxidant activity of the aqueous fraction aligns with its higher phenolic and flavonoid contents, highlighting the greater extraction efficiency of polar solvents for antioxidant phytochemicals (Shahidi and Ambigaipalan, 2015; Panche *et al.*, 2016).

Antioxidant potential of plant extracts can be linked to the phenolics within the extract because of the phenolic acids

(Shi *et al.*, 2022). These are involved in reactions with ROS, such as hydroxyl, superoxide, and lipid peroxyl radicals, which protect lipids from oxidation (Platzer *et al.*, 2022). Scavenging activity is linked to the number of hydroxyl groups, which provide hydrogen atoms to the radicals.

IC₅₀ measures the potency of an agent as an antioxidant. IC₅₀ is the concentration required to scavenge 50% of DPPH radicals. Therefore, a lower IC₅₀ means higher antioxidant potency. The IC₅₀ values recorded for AF, CF, and ME were 79.75, 87.36, and 116.41 µg/mL, respectively (Figure 3).

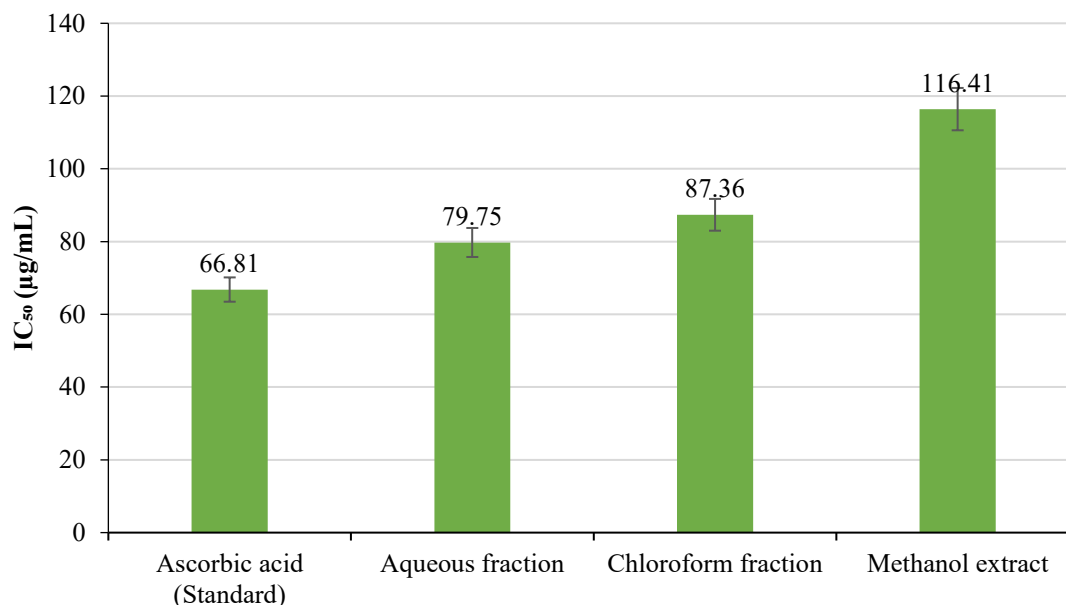


Fig. 3 IC₅₀ (µg/mL) Values of *H. latifolia* Extracts and Fractions Compared with Ascorbic Acid Standard

3.6. Ferric ion Reducing Antioxidant Potential

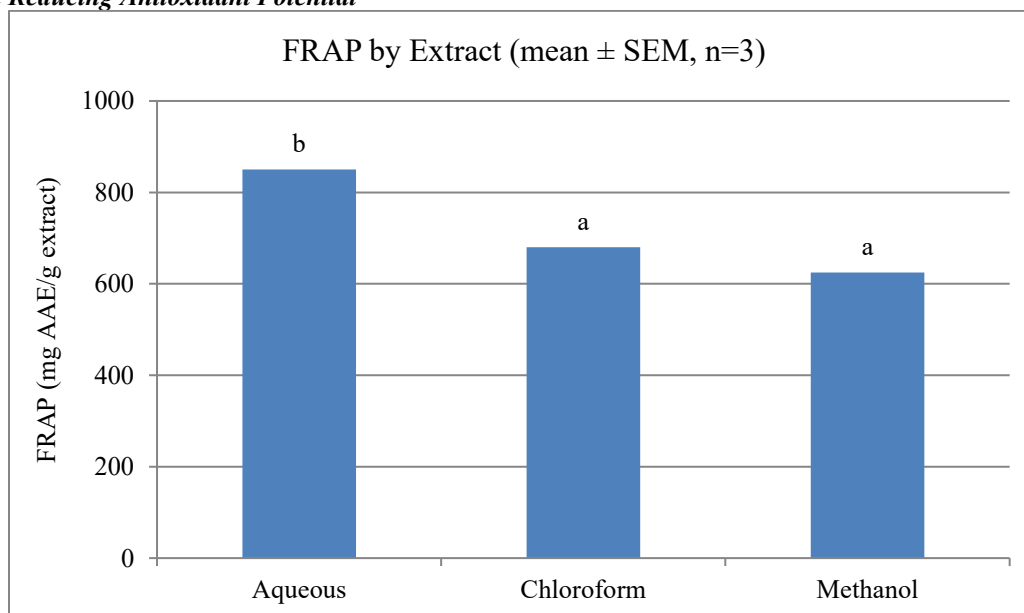


Fig. 4 Ferric reducing antioxidant power (FRAP) of *H. latifolia* samples (mean ± SEM, n = 3). Bars with different superscript letters differ significantly at $p < 0.05$ according to one-way ANOVA followed by Tukey's post hoc test

FRAP is an Electron-Transfer (ET)-based antioxidant assay that determines the capability of a substance to reduce the Fe^{3+} -TPTZ to Fe^{2+} -TPTZ complex under acidic conditions (Shi *et al.*, 2022). According to the current experiment, the AF showed the highest amount of FRAP (853.05 ± 12.80 mg AAE/g), followed by the CF (683.70 ± 22.17 mg AAE/g) and ME (625.64 ± 8.38 mg AAE/g) (Figure 4). The results obtained indicate that AF possessed higher reducing power compared to the other extracts ($p < 0.05$). Nevertheless, the difference between the CF and ME is also significant, which means that both extracts possess similar reducing capabilities.

Such results point to the fact that the antioxidant activity of *H. latifolia* might mainly depend on the presence of polar phytochemicals, as they possess enhanced electron-donating ability as well as reducing ability. Higher FRAP values in the AF may be due to higher amounts of phenolics and flavonoids present in the AF, which in turn might have helped to improve the antioxidant potential (Shahidi and Ambigaipalan, 2015). Similar observations have been made by Ajayi *et al.* (2024), wherein greater FRAP activity was noted for the polar fractions of *H. latifolia* than for the non-polar fractions.

3.7. Correlation Between Polyphenolic Content and DPPH Antioxidant Activity (IC_{50})

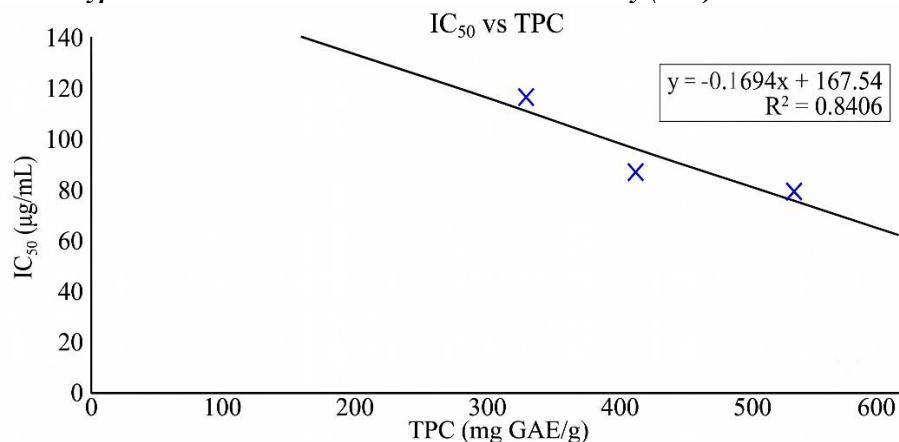


Fig. 5A Correlation between TPC and DPPH scavenging activity (IC_{50}) in extract and fractions of *H. latifolia* samples

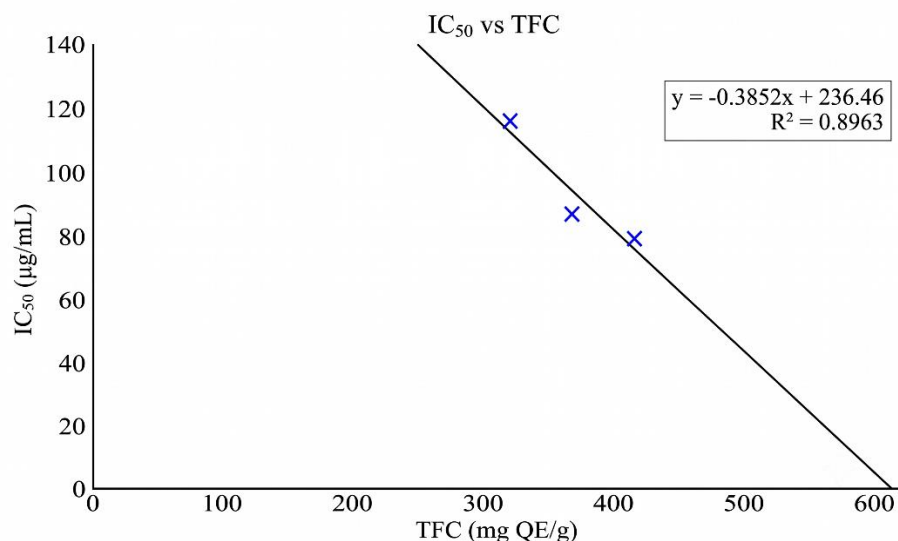


Fig 5B. Correlation between TFC and DPPH scavenging activity (IC_{50}) in extract and fractions of *H. latifolia*.

From Figure 5A, it is evident that there was a negative correlation between IC_{50} and TPC ($y = -0.1694x + 167.54$; $R^2 = 0.8406$) and a strong negative correlation between IC_{50} and TFC ($y = -0.3852x + 236.46$; $R^2 = 0.8963$) from Figure 5B. These inverse correlations demonstrate that with increasing phenolic and flavonoid contents, there is a

corresponding decrease in IC_{50} values, leading to an increase in antioxidant activity in this particular study. This confirms the significance of phenolic compounds as potent electron or hydrogen donors responsible for breaking the free-radical chain reactions (Shahidi and Ambigaipalan, 2015; Kruk, 2022). In addition, the relatively higher value of R^2 in TFC

may imply that flavonoids play a significant role in the radical scavenging activity of *H. latifolia* compared to the phenolic compound class in general. This might be due to the nature of the structure of the flavonoids, which includes the presence of conjugated double bonds and hydroxyl groups in aromatic rings, increasing their redox activity and reactivity towards reactive oxygen species (Kumar and Pandey, 2013; Panche *et al.*, 2016; Platzer *et al.*, 2022). Hence, it can be suggested that the high antioxidant potential of *H. latifolia* is attributed to its flavonoid content along with other phenolic compounds (Shahidi and Ambigaipalan, 2015).

4. Conclusion

This work demonstrates that *H. latifolia* (whole plant) has appreciable proximate profiles and mineral contents, with a low Na: K ratio. It also indicates that the plant has high levels of phenolics and flavonoids, especially in the AF. Its

antioxidant capacity, measured by DPPH and FRAP, correlates with phenolic/flavonoid content (AF > CF > ME). These data support the plant's nutritional and antioxidant potential to combat various diseases caused by free radicals. Further studies are underway to isolate, identify, characterize, and elucidate the structures of the bioactive compounds.

Conflict of Interest

No conflict of interest declared by the authors.

Authors' Declaration

It has been declared by the authors that the current manuscript is original, and they take full responsibility for the content of the article.

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