

# Chemical Composition and Phytochemical Profiling of *Phyllanthus Niruri* Aqueous Leaf Extract

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**Abstract-** This study investigated the chemical composition, phytochemical constituents, mineral profile, and vitamin composition of *Phyllanthus niruri* aqueous leaf extract using standard analytical techniques. Gas chromatography–mass spectrometry (GC-MS) analysis showed the presence of several bioactive volatile compounds, including 11-octadecanoic acid methyl ester (33.38%), 9,12-octadecadienoic acid methyl ester (21.45%), hexadecanoic acid methyl ester (14.61%), and 1-octadecene (8.78%). Qualitative phytochemical screening confirmed the presence of flavonoid, alkaloid, phenol, tannin, saponin, and curcumin. Quantitative phytochemical analysis showed alkaloid as the most abundant phytochemical constituent ( $68.76 \pm 0.49$  mg/g), phenol ( $57.70 \pm 0.42$  mg/g) and flavonoid ( $30.46 \pm 0.08$  mg/g) respectively. Mineral analysis indicated that calcium possessed the highest concentration ( $9.04 \pm 0.02$  ppm), while sodium had the lowest value ( $0.47 \pm 0.01$  ppm). Water-soluble vitamin analysis revealed that vitamin C was the most abundant ( $49.32 \pm 0.00$  mg/L), whereas vitamin E exhibited the highest concentration among fat-soluble vitamins ( $91.74 \pm 0.01$  mg/L). Proximate analysis showed high protein content ( $64.71 \pm 0.44\%$ ), indicating the nutritional significance of the plant. The findings suggest that *P. niruri* aqueous leaf extract is rich in essential bioactive compounds and nutrients that may contribute to its medicinal efficacy and antioxidant properties. Therefore, *P. niruri* possesses strong potential for pharmaceutical, nutraceutical, and therapeutic applications.

**Keywords:** *Phyllanthus Niruri*, Phytochemicals, GC-MS, Minerals, Vitamins, Aqueous Leaf Extract, Antioxidants.

## I. INTRODUCTION

Medicinal plants have continued to attract significant scientific interest due to their diverse therapeutic potentials and their importance in traditional medicine systems worldwide. Plants synthesize a wide range of biologically active compounds known

as phytochemicals, which contribute to their medicinal, antimicrobial, antioxidant, anti-inflammatory, and pharmacological properties. These bioactive compounds have gained considerable attention because of their applications in drug development, nutraceutical production, and disease management (Bagalkotkar et al., 2006).

Among medicinal plants, *Phyllanthus niruri*, commonly referred to as “stone breaker,” belongs to the family Euphorbiaceae and is widely distributed in tropical and subtropical regions. The plant has been extensively utilized in traditional medicine for the treatment of kidney stones, liver disorders, urinary tract infections, diabetes, hypertension, jaundice, and inflammatory diseases (Narendra et al., 2012). Previous studies have also reported that *P. niruri* exhibits antiviral, antibacterial, antioxidant, hepatoprotective, antidiabetic, and anti-inflammatory activities owing to its rich phytochemical composition (Harish & Shivanandappa, 2006).

The therapeutic properties of *P. niruri* have been linked to the presence of important secondary metabolites such as alkaloids, flavonoids, tannins, phenolic compounds, lignans, glycosides, saponins, and terpenoids (Kaur et al., 2017). These phytochemicals possess strong antioxidant activities capable of scavenging free radicals and reducing oxidative stress within biological systems. In addition, the plant contains essential minerals and vitamins that contribute to its nutritional and pharmacological importance.

Gas chromatography–mass spectrometry (GC-MS) has emerged as one of the most reliable analytical techniques for identifying volatile and semi-volatile

phytochemical constituents in medicinal plants. Similarly, quantitative and qualitative phytochemical analyses are essential in determining the medicinal value and therapeutic relevance of plant extracts. Evaluation of mineral and vitamin composition further provides important information regarding the nutritional significance of medicinal plants.

Despite the increasing medicinal applications of *P. niruri*, there remains limited information on the comprehensive chemical composition of its aqueous leaf extract, particularly regarding its volatile phytochemical profile, vitamin composition, and mineral constituents. Therefore, this study was designed to investigate the chemical composition, phytochemical constituents, mineral profile, and vitamin composition of *Phyllanthus niruri* aqueous leaf extract using GC-MS and standard phytochemical analytical methods.

## II. MATERIALS AND METHOD

### 2.1 Collection and Identification of Plant Material

Fresh leaves of *Phyllanthus niruri* were collected within the premises of the school of Industrial & Applied sciences, Federal Polytechnic Nekede, Owerri and transported to the laboratory for analysis. The plant material was authenticated based on its morphological characteristics before use. The leaves were washed thoroughly with distilled water to remove adhering dirt and contaminants and then air-dried for 7 days at room temperature to preserve the phytochemical constituents. The dried leaves were pulverized into fine powder using a laboratory blender and stored in airtight containers until extraction and analysis were performed (Kaur et al., 2017).

### 2.2 Preparation of Aqueous Leaf Extract

The pulverized leaves of *P. niruri* were extracted using distilled water according to the method described by Harborne (1998) with slight modifications. A 50 g of sample was soaked in 250mL of deionized water and kept boiling at 100°C for 30 minutes. After, the extract was cooled at room temperature then filtered using a Whatman No.1 filter paper. The filtrate obtained was stored at 4°C for further studies.

### 2.3 Determination of chemical composition

#### 2.3.1 GC-MS Analysis of Phytochemical Constituents

Gas chromatography–mass spectrometry (GC-MS) analysis was carried out using a combined 7890A gas chromatograph system coupled with a mass spectrophotometer fitted with HP-5 MS fused silica column (30.0 m × 250 µm; film thickness 0.25 µm). Helium gas served as the carrier gas with a flow rate of 1.0 mL/min. Identification of compounds was achieved by comparing their mass spectra with standard spectra from the National Institute of Standards and Technology (NIST) database (Regis Correa da Silva, 2024).

#### 2.3.2 Qualitative Phytochemical Analysis

Qualitative screening and Quantitative phytochemical were carried out using standard analytical spectrophotometric methods described by Sofowora (2008), Trease and Evans (2009), and Harborne (1998). The phytochemicals analyzed included flavonoids, alkaloids, phenols, tannins, saponins, and curcumin.

#### 2.3.3 Proximate composition Analysis

Proximate analysis of *P. niruri* aqueous leaf extract was carried out to determine moisture, ash, crude fibre, crude fat, crude protein, and carbohydrate contents using the standard procedures of the Association of Official Analytical Chemists (AOAC, 2016). Moisture content was determined by oven drying to constant weight, ash content by incineration in a muffle furnace at 550°C, crude fat using Soxhlet extraction method, crude fibre by acid and alkali digestion, and crude protein by Kjeldahl digestion method. Carbohydrate content was estimated by difference (AOAC, 2016).

#### 2.3.4 Determination of Mineral Composition

Mineral analysis was carried out using Atomic Absorption Spectrophotometry (AAS) according to AOAC (2016) methods. The minerals analyzed included sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), and zinc (Zn). Results obtained were expressed in parts per million (ppm).

#### 2.3.5 Determination of Water-Soluble Vitamins

Water-soluble vitamins including vitamins B1, B2, B3, B6, B12, and vitamin C were determined using

standard spectrophotometric analytical procedures as described by AOAC (2016).

### 2.3.6 Determination of Fat-Soluble Vitamins

Fat-soluble vitamins including vitamins A, D, E, and K were analyzed using solvent extraction and spectrophotometric methods according to AOAC (2016).

### 2.4 Statistical Analysis

All experiments were carried out in triplicates, and results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA), while Tukey post hoc test was used for comparison of means using GraphPad Prism statistical software. Statistical significance was accepted at  $p \leq 0.05$ .

## III. RESULTS

### 3.1 GC-MS Phytochemical Composition of Phyllanthus niruri Aqueous Leaf Extract

The GC-MS analysis of Phyllanthus niruri aqueous leaf extract revealed the presence of several volatile bioactive compounds with varying retention times and percentage compositions.

Among the detected phytochemicals, 11-octadecanoic acid methyl ester represented the highest percentage composition (33.38%), followed by 9,12-octadecadienoic acid methyl ester (21.45%), hexadecanoic acid methyl ester (14.61%), and 1-octadecene (8.78%). Other compounds identified included cyclooctane methyl, acetic acid n-octadecyl ester, heptadecyl heptafluorobutyrate, trichloroacetic acid tetradecyl ester, and oxirane derivatives.

The abundance of fatty acid methyl esters observed in the extract suggests the presence of potent antioxidant and antimicrobial constituents within P. niruri. These compounds are known to exhibit anti-inflammatory, antimicrobial, hepatoprotective, and free radical scavenging activities.

Table 3.1 GC-MS Phytochemical Composition of Phyllanthus niruri Aqueous Leaf Extract

S/N	Compound Name	Retention Time (min)	Molecular Formula	Molecular Weight	Percentage (%)
1	Hexadecanoic acid, methyl ester	15.62	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270	14.61
2	1-Octadecene	18.74	C <sub>18</sub> H <sub>36</sub>	252	8.78
3	9,12-Octadecadienoic acid, methyl ester	21.56	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	294	21.45
4	11-Octadecanoic acid, methyl ester	24.33	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	296	33.38
5	Cyclooctane methyl	12.81	C <sub>9</sub> H <sub>18</sub>	126	4.62
6	Acetic acid, n-octadecyl ester	9.47	C <sub>20</sub> H <sub>40</sub> O <sub>2</sub>	312	5.12
7	Heptadecyl heptafluorobutyrate	27.62	C <sub>21</sub> H <sub>35</sub> F <sub>7</sub> O <sub>2</sub>	474	3.85
8	Trichloroacetic acid tetradecyl ester	31.08	C <sub>16</sub> H <sub>29</sub> Cl <sub>3</sub> O <sub>2</sub>	359	4.19
9	Oxirane derivative	6.23	C <sub>18</sub> H <sub>36</sub> O	268	2.76

S/N	Phytochemical Constituent	Test Used	Observation	Inference
1.	Flavonoids	Alkaline reagent test	Yellow coloration	Present (+)
2.	Alkaloids	Wagner's test	Reddish-brown precipitate	Present (+)
3.	Phenols	Ferric chloride test	Black-black coloration	Present (+)
4.	Tannins	Ferric chloride test	Greenish-black coloration	Present (+)
5.	Saponins	Froth test	Persistent froth	Present (+)
6.	Curcumin	NaOH test	Reddish-brown coloration	Present (+)

(+) = Present (-) = Absent

fig 3.1 Qualitative Phytochemical Composition of Phyllanthus niruri Aqueous Leaf Extract

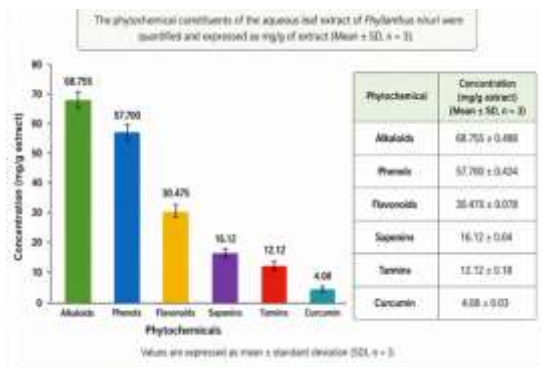


Fig 3.2 Quantitative Phytochemical Composition of Phyllanthus niruri Aqueous Leaf Extract

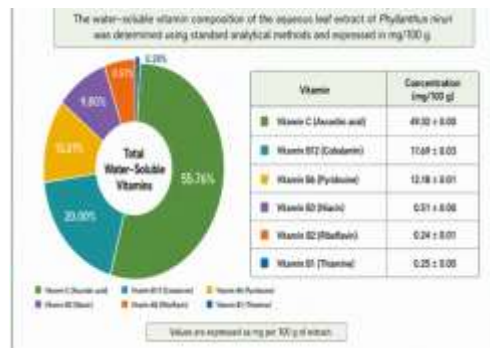


Fig 3.6 Fat-Soluble Vitamins Composition of Phyllanthus niruri Aqueous Leaf Extract

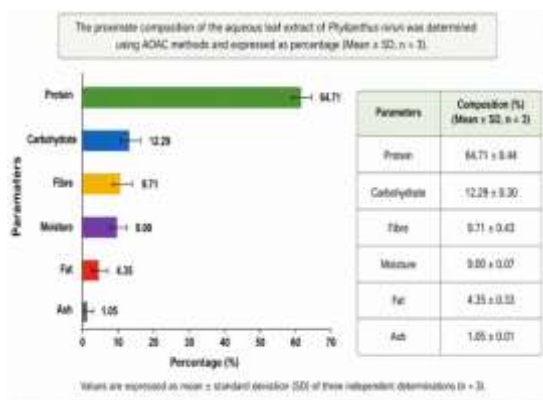
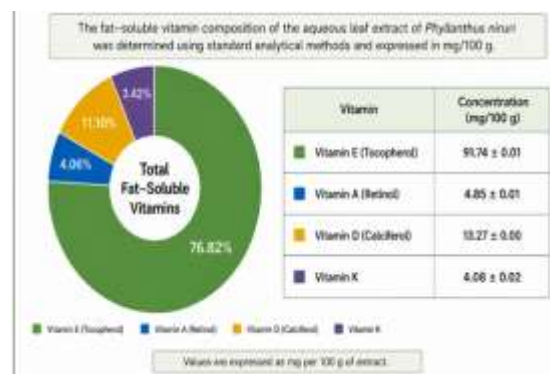


Fig 3.4 Mineral Composition of Phyllanthus niruri Aqueous Leaf Extract



#### IV. DISCUSSION

The present study investigated the chemical composition, phytochemical constituents, mineral profile, and vitamin composition of Phyllanthus niruri aqueous leaf extract. The findings revealed that the plant possesses numerous bioactive compounds and essential nutrients which may account for its wide therapeutic applications in traditional medicine. The GC-MS analysis demonstrated the presence of several volatile phytochemicals, particularly fatty acid methyl esters and hydrocarbon derivatives. Among the identified compounds, 11-octadecanoic acid methyl ester recorded the highest percentage composition, followed by 9,12-octadecadienoic acid methyl ester and hexadecanoic acid methyl ester. These compounds have been previously associated with antioxidant, antimicrobial, anti-inflammatory, and hepatoprotective activities. Fatty acid esters are known to stabilize cellular membranes and may contribute to the free radical scavenging activities of medicinal plants (Bagalkotkar et al., 2006). The abundance of these phytochemicals in *P. niruri* supports the medicinal importance of the plant and

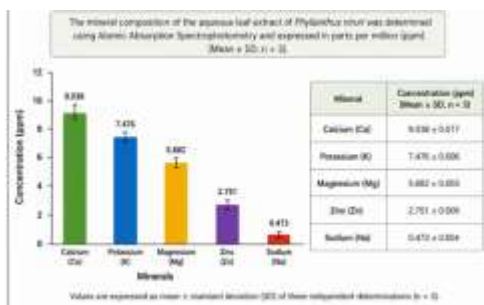


Fig 3.5 Water-Soluble Vitamins Composition of Phyllanthus niruri Aqueous Leaf Extract

may explain its traditional use in the management of inflammatory and infectious diseases.

Qualitative phytochemical screening confirmed the presence of flavonoids, alkaloids, phenols, tannins, saponins, and curcumin in the aqueous leaf extract. These phytochemicals are biologically important secondary metabolites known for their therapeutic and pharmacological activities. Flavonoids and phenolic compounds possess strong antioxidant properties capable of scavenging reactive oxygen species and reducing oxidative stress within biological systems. Alkaloids are recognized for their antimicrobial, analgesic, antihypertensive, and anticancer effects, while tannins and saponins contribute to antimicrobial and anti-inflammatory activities (Narendra et al., 2012). The presence of these phytochemicals therefore indicates that *P. niruri* possesses significant medicinal and pharmaceutical potentials.

Quantitative phytochemical analysis revealed that alkaloids had the highest concentration among the detected phytochemicals, followed closely by phenols and flavonoids. The high alkaloid content observed in this study may contribute significantly to the therapeutic efficacy of *P. niruri*, particularly in the treatment of microbial infections and inflammatory conditions. Alkaloids have been reported to exhibit diverse pharmacological properties including antimicrobial, antihypertensive, antimalarial, and antitumor activities (Akinpelu & Onakoya, 2006). Similarly, the elevated phenolic and flavonoid contents suggest strong antioxidant activities of the plant extract. Phenolic compounds are capable of donating hydrogen atoms to neutralize free radicals, thereby preventing lipid peroxidation and cellular damage. The antioxidant properties of flavonoids and phenols may also contribute to the hepatoprotective and nephroprotective functions previously attributed to *P. niruri* (Harish & Shivanandappa, 2006).

Mineral analysis revealed the presence of essential minerals including calcium, potassium, magnesium, zinc, and sodium. Calcium recorded the highest concentration among the analyzed minerals. Calcium plays an important role in bone formation, muscle contraction, nerve impulse transmission, and blood

clotting. Potassium and magnesium are important intracellular electrolytes involved in maintaining osmotic balance, nerve function, and enzymatic reactions. Zinc is an essential trace element known for its immune-boosting, antioxidant, and wound-healing functions. The presence of these minerals indicates that *P. niruri* may contribute significantly to physiological and metabolic activities within the body. The low sodium concentration observed may also be beneficial in reducing the risk of hypertension and cardiovascular complications associated with excessive sodium intake.

The analysis of water-soluble vitamins revealed that vitamin C had the highest concentration among the vitamins detected. Vitamin C is a potent antioxidant that protects cells against oxidative damage by scavenging free radicals. It also plays important roles in collagen synthesis, immune function, wound healing, and iron absorption. The high concentration of vitamin C observed in this study may therefore contribute significantly to the antioxidant and immune-enhancing properties of *P. niruri*. Furthermore, the presence of B-complex vitamins such as vitamins B1, B2, B3, B6, and B12 indicates the nutritional importance of the plant in supporting cellular metabolism, nervous system function, and energy production.

Among the fat-soluble vitamins analyzed, vitamin E recorded the highest concentration. Vitamin E is a powerful lipid-soluble antioxidant known for protecting cell membranes against oxidative damage caused by reactive oxygen species. It also contributes to immune regulation and prevention of degenerative diseases. The high concentration of vitamin E in *P. niruri* further supports the strong antioxidant potential of the plant extract. Vitamins A, D, and K identified in the extract are also essential for vision, calcium metabolism, immune function, and blood coagulation respectively. These findings suggest that *P. niruri* possesses substantial nutritional and therapeutic benefits attributable to its rich vitamin composition.

## CONCLUSION

The findings of the present study demonstrated that *Phyllanthus niruri* aqueous leaf extract contains abundant phytochemicals, essential minerals,

vitamins, and nutritional constituents that support its therapeutic importance in traditional and modern medicine. The GC-MS analysis revealed the presence of several bioactive volatile compounds, including fatty acid methyl esters such as 11-octadecanoic acid methyl ester, 9,12-octadecadienoic acid methyl ester, and hexadecanoic acid methyl ester. These findings are consistent with earlier reports by Bagalkotkar et al. (2006), who reported that *P. niruri* contains several biologically active phytoconstituents responsible for its antioxidant and antimicrobial activities. The qualitative and quantitative phytochemical analyses confirmed the presence of flavonoids, alkaloids, phenols, tannins, saponins, and curcumin, with alkaloids and phenols occurring in the highest concentrations. Similar observations were reported by Narendra et al. (2012) and Kaur et al. (2017), who documented that *P. niruri* is rich in alkaloids, flavonoids, lignans, and phenolic compounds which contribute significantly to its pharmacological properties. The high phenolic and flavonoid contents observed in this study further support the findings of Harish and Shivanandappa (2006), who associated the antioxidant potential of *P. niruri* with its phenolic-rich composition.

#### REFERENCES

- [1] Bagalkotkar, G., Sagineedu, S. R., Saad, M. S., & Stanslas, J. (2006). Phytochemicals from *Phyllanthus niruri* Linn. and their pharmacological properties: A review. *Journal of Pharmacy and Pharmacology*, 58(12), 1559–1570.
- [2] Narendra, K., Swathi, J., Sowjanya, K. M., & Satya, A. K. (2012). *Phyllanthus niruri*: A review on its ethnopharmacological, phytochemical and pharmacological profile. *Journal of Pharmacy Research*, 5(9), 4681–4691.
- [3] Harish, R., & Shivanandappa, T. (2006). Antioxidant activity and hepatoprotective potential of *Phyllanthus niruri*. *Food Chemistry*, 95(2), 180–185.
- [4] Kaur, N., Kaur, B., & Sirhindi, G. (2017). *Phyllanthus niruri*: A review on its ethnobotanical, phytochemical and pharmacological profile. *Journal of Pharmacognosy and Phytochemistry*, 6(4), 468–473.
- [5] Harborne, J. B. (1998). *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis* (3rd ed.). Chapman and Hall, London.
- [6] AOAC. (2016). *Official Methods of Analysis of the Association of Official Analytical Chemists* (20th ed.). Washington DC, USA.
- [7] Sofowora, A. (2008). *Medicinal Plants and Traditional Medicine in Africa* (3rd ed.). Spectrum Books Ltd., Ibadan.
- [8] Trease, G. E., & Evans, W. C. (2009). *Pharmacognosy* (16th ed.). Saunders Elsevier, London.
- [9] Zhishen, J., Mengcheng, T., & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry*, 64(4), 555–559.
- [10] Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, 16, 144–158.
- [11] Pearson, D. (1976). *The Chemical Analysis of Foods* (7th ed.). Churchill Livingstone, Edinburgh.
- [12] Obadoni, B. O., & Ochuko, P. O. (2001). Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. *Global Journal of Pure and Applied Sciences*, 8(2), 203–208.
- [13] Regis Correa da Silva, D. (2024). GC-MS analytical procedures for phytochemical determination. *Analytical Chemistry Methods*, 14(3), 221–230.
- [14] Akinpelu, D. A., & Onakoya, T. M. (2006). Antimicrobial activities of medicinal plants used in folklore remedies in south-western Nigeria. *African Journal of Biotechnology*, 5(11), 1078–1081.
- [15] Olaleye, M. T. (2007). Cytotoxicity and antibacterial activity of methanolic extract of *Phyllanthus niruri*. *Journal of Medicinal Plants Research*, 1(3), 26–30.