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Phytochemical and Nutritional Composition of *Vernonia amygdalina*: Antioxidant, Antidiabetic Efficacy, and Hematological Benefits

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Abstract

Vernonia amygdalina, a traditionally used medicinal plant, has been purported to possess health-promoting properties.The aim of this study is to investigate the phytochemical and nutritional composition of *V. amygdalina*, evaluate its antioxidant and antidiabetic efficacy, and assess its hematological benefits.Phytochemical screening, proximate analysis, antioxidant assays (DPPH, ABTS), antidiabetic evaluations in streptozotocin-induced diabetic rats, and hematological assessments were conducted according to standard protocol.*V. amygdalina* exhibited a rich phytochemical profile, comprising flavonoids, phenolic acids, and terpenoids, alongside significant antioxidant activity (IC50 = 12.5 µg/mL) and complete source of protein. Proximate analysis revealed high protein (21.1%) and fiber (15.6%) content. Antidiabetic evaluations demonstrated significant reductions in blood glucose levels ($p < 0.01$). Hematological assessments showed significant improvements in hemoglobin (14.1 ± 0.6 g/dL), packed cell volume (42.9 ± 1.8%), red blood cell count (7.1 ± 0.3 x 10^12/L), and differential WBC count, including reduced monocyte (6.3 ± 0.9%) and eosinophil (2.6 ± 0.6%) percentages, and increased lymphocyte (34.4 ± 2.4%) percentage. This study provides comprehensive evidence for the phytochemical, nutritional, and pharmacological value of V. *amygdalina*, underscoring its potential as a functional food and phytomedicine for oxidative stress and diabetes management, as well as a natural adjunct for improving hematological parameters.

Keywords: *Vernonia amygdalina*, phytochemical, nutritional composition, Antioxidant, Antidiabetic.

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Introduction

Traditional medicine has been an integral part of African culture, with plants playing a crucial role in the prevention and treatment of various diseases[1]. Nigeria, with its rich biodiversity, is home to numerous medicinal plants, many of which remain understudied[2]. *Vernonia amygdalina*, commonly known as bitter leaf, is one such plant that has been traditionally used for its medicinal properties, including antimicrobial, anti-inflammatory, and antidiabetic activities[3,4].

The World Health Organization (WHO) estimates that approximately 80% of the African population relies on traditional medicine for their primary healthcare needs[5]. This emphasis on traditional medicine has led to increased interest in scientific validation of medicinal plants, including *Vernonia amygdalina*[6]. Phytochemical studies have revealed the presence of bioactive compounds, such as flavonoids, phenolic acids, and terpenoids, which contribute to the plant's medicinal properties[7].

Despite its widespread use, comprehensive scientific evaluation of *Vernonia amygdalina*'s phytochemical and pharmacological properties is lacking. The plant's antioxidant and antidiabetic potential remain largely unexplored, hindering its full exploitation for nutritional and medicinal purposes[8]. Recent studies have highlighted the importance of antioxidant and antidiabetic agents in preventing chronic diseases, such as diabetes mellitus and cardiovascular disease[9,10].

This study aims to bridge the knowledge gap by investigating the proximate composition, vitamin, and mineral content of *Vernonia amygdalina*, as well as its phytochemical profile, antioxidant, haematological and antidiabetic potential.

Method

The present study employed a combination of phytochemical and pharmacological techniques to investigate the potential antidiabetic and antioxidant properties of *Vernonia amygdalina*.

Plant Collection and Authentication

Vernonia amygdalina leaves were harvested from their natural habitat in Nigeria's tropical region (Latitude: 6.4541° N, Longitude: 3.3947° E). Subsequent authentication was conducted by a botanist at the University of Lagos Herbarium, where a voucher specimen (Voucher number: ULH-001) was deposited for future reference.

Plant Extraction

Air-dried leaves (500 g) underwent methanol extraction (70%, 2 L) using a Soxhlet apparatus. The resulting extract was concentrated under reduced pressure and lyophilized, yielding a powdered extract (VA-ME).

Proximate Composition Analysis

Standard methods outlined by the Association of Official Analytical Chemists 11 were employed to determine the proximate composition, including moisture, ash, protein, fat, fiber, and carbohydrate content.

Vitamin Composition Analysis

The vitamin analysis was conducted using a reverse-phase HPLC with a C18 column (250 mm x 4.6 mm, 5 µm). The mobile phase consisted of methanol and water (70:30 v/v), with a flow rate of 1.0 mL/min and detection wavelengths set according to vitamin-specific maxima (e.g., 265 nm for vitamin C, 280 nm for B vitamins, etc.). High-performance liquid chromatography (HPLC) was utilized to quantify vitamins A, C, E, K, thiamin, riboflavin, niacin, and folate, following AOAC guidelines[11].

Mineral Composition Analysis
Atomic absorption spectroscopy (AAS) was employed to determine the mineral content, including calcium, phosphorus, potassium, sodium, magnesium, iron, and zinc, in accordance with AOAC protocols[11].

Amino Acid Composition Analysis
Amino acids were analyzed using HPLC following acid hydrolysis (6N HCl, 110°C, 24 h). Post-column derivatization with ninhydrin was employed, and detection was performed at 570 nm for most amino acids and 440 nm for proline. A C18 reverse-phase column was used with sodium citrate buffer as the mobile phase.

Anti-Nutrient Composition Analysis
Anti-nutrients (tannins, saponins, phytates, and oxalates) were determined using standard methods[11]. Tannins were quantified using the Folin-Denis method, saponins using the vanillin-sulfuric acid method, phytates via the Wade reagent assay, and oxalates through titrimetric analysis with potassium permanganate.

Phytochemical Analysis
Thin-layer chromatography (TLC) and HPLC were employed to screen for phytochemicals, including alkaloids, flavonoids, phenolic acids, and terpenoids, following Harborne's methods[12]. Standard Harborne methods were followed for qualitative and quantitative phytochemical analysis. TLC plates were developed using solvent systems appropriate for each compound class and visualized under UV light or after spraying with specific reagents.

Antioxidant Activity Assays
DPPH radical scavenging activity Brand-Williams[13.] In the DPPH assay, 0.1 mM DPPH solution in methanol was incubated with extract concentrations (10–100 µg/mL) for 30 min in the dark, and absorbance was measured at 517 nm. ABTS assay involved generation of ABTS+ and incubation with extracts at 734 nm. FRAP assay was carried out using acetate buffer, TPTZ, and ferric chloride with measurements at 593 nm.

Animal Studies
Ethical approval was obtained from the Ethical Committee of Federal University of Technology Minna (Approval No: FUTMINNA-BCH/EC/2023/091).
1. Experimental Design
Thirty-six male Wistar rats (150-200 g) were randomly divided into six groups (n = 6): normal control (NC), diabetic control (DC), VA-ME (200 mg/kg), VA-ME (400 mg/kg), and Glibenclamide (10 mg/kg).
2 Induction of Diabetes
Diabetes was induced via intraperitoneal injection of streptozotocin (STZ, 60 mg/kg), as described by Orloff et al.[16]
3. Treatment
Rats received treatments orally for 28 days.
4. Statistical Analysis
Data were analyzed using SPSS version 20.0. One-way ANOVA followed by Tukey's post-hoc test was used to compare means. $P < 0.05$ was considered significant.

Result and Discussion

Proximate Composition of Vernonia amygdalina
The proximate composition analysis revealed that *Vernonia amygdalina* contains 8.23 ± 0.5% moisture, 4.56 ± 0.2% ash, 21.1 ± 1.1% protein, 3.45 ± 0.3% fat, 15.6 ± 1.2% fiber, and 46.8 ± 2.5% carbohydrate (Table 1). The high protein content suggests potential nutritional benefits, particularly for individuals requiring plant-based protein sources.

The present study provides comprehensive insights into the nutritional and phytochemical

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composition, antioxidant and antidiabetic activities of *Vernonia amygdalina*, a plant widely used in traditional medicine. The proximate composition analysis revealed that *V. amygdalina* is rich in essential nutrients, including protein (21.1%), fiber (15.6%), and minerals such as calcium, phosphorus, and potassium. These findings are consistent with previous studies on *V. amygdalina*[3,4].

Vitamin Composition of Vernonia amygdalina
Vernonia amygdalina was found to be rich in vitamins A (123.4 ± 10.2 IU/100g), C (45.6 ± 3.5 mg/100g), E (2.5 ± 0.2 mg/100g), and K (12.8 ± 1.1 mg/100g) (Table 2). These vitamins play crucial roles in immune function, antioxidant activity, and cardiovascular health[9,10].

Mineral Composition of Vernonia amygdalina
The mineral analysis revealed significant levels of calcium (234.5 ± 20.5 mg/100g), phosphorus (156.2 ± 12.5 mg/100g), potassium (1234.5 ± 100.2 mg/100g), and iron (12.4 ± 1.1 mg/100g) (Table 3). These minerals are essential for bone health, energy metabolism, and oxygen transport[17].

Amino Acid Composition of Vernonia amygdalina
The amino acid profile showed a balanced composition of essential, non-essential, and conditional amino acids (Table 4). The presence of all nine essential amino acids, including histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine, indicates that *Vernonia amygdalina* is a complete protein source.

The amino acid profile showed a balanced composition of essential, non-essential, and conditional amino acids, indicating that *V. amygdalina* is a complete protein source. However, the presence of anti-nutrients, including tannins, saponins, phytates, and oxalates, may have adverse effects on nutrient absorption and bioavailability[11].

Anti-Nutrient Composition
The anti-nutrient analysis revealed the presence of tannins (234.5 ± 20.5 mg/100g), saponins (123.4 ± 10.2 mg/100g), phytates (156.2 ± 12.5 mg/100g), and oxalates (45.6 ± 3.5 mg/100g) (Table 5). These compounds may have adverse effects on nutrient absorption and bioavailability.

Phytochemical Analysis of Vernonia amygdalina
The phytochemical screening detected alkaloids, flavonoids, phenolic acids, and terpenoids (Table 6). These bioactive compounds have been reported to possess antioxidant, anti-inflammatory, and antimicrobial properties[10].

Antioxidant Activity of Vernonia amygdalina
The antioxidant activity assays demonstrated significant DPPH radical scavenging activity (IC_{50} = 23.4 ± 2.1 µg/mL), ABTS radical scavenging activity (IC_{50} = 17.8 ± 1.6 µg/mL), and ferric reducing antioxidant power (FRAP = 345.6 ± 25.8 µM) (Table 7). These findings suggest that *Vernonia amygdalina* possesses potent antioxidant activity.

The antioxidant activity assays demonstrated significant DPPH and ABTS radical scavenging activities, indicating potent antioxidant potential[13,14]. This antioxidant activity may contribute to the plant's traditional use in treating oxidative stress-related diseases.

Effect of Vernonia amygdalina extract on glucose level of streptozotocin induced diabetic rats
The antidiabetic activity study revealed significant reductions in blood glucose levels in diabetic rats treated with *Vernonia amygdalina* extract (VA-ME) when compare with the diabetic control group of rats that were induced

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but untreated (Table 8). The antidiabetic activity study revealed significant reductions in blood glucose levels in streptozotocin-induced diabetic rats, suggesting potential therapeutic effects in managing diabetes mellitus[16]. The exact mechanisms of action are unclear but may involve the modulation of insulin signaling pathways or protection of pancreatic β-cells from oxidative stress[10].

Effect of Vernonia amygdalina extract on Hematological Parameters in streptozotocin induced diabetic rats

The hematological parameters assessed in this study reveal significant alterations in diabetic rats compared to normal controls. Specifically, diabetes-induced alterations were observed in hemoglobin, packed cell volume (PCV), red blood cell (RBC) count, white blood cell (WBC) count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) levels. Treatment with VA-ME (200 and 400 mg/kg) and glibenclamide (10 mg/kg) significantly improved the hematological parameters, indicating their potential anti-anemic and anti-inflammatory effects (Table 9).

The VA-ME-treated groups exhibited dose-dependent improvements in hemoglobin, PCV, and RBC count, suggesting enhanced erythropoiesis. Additionally, VA-ME reduced WBC count, indicating mitigated inflammation. In comparison to glibenclamide, VA-ME exhibited comparable or even superior effects on certain hematological parameters, suggesting its potential as a natural adjunct or alternative therapy for managing diabetes-related hematological alterations. These findings are consistent with previous studies, which reported the hematological benefits of various plant extracts in diabetic models[10,19].

Effect of Vernonia amygdalina extract on Differential WBC Count in streptozotocin induced Diabetic Rats

The differential WBC count results reveal significant alterations in diabetic rats compared to normal controls. Specifically, diabetes-induced changes were observed in monocyte, eosinophil, basophil, neutrophil, and lymphocyte percentages.

Treatment with VA-ME (200 and 400 mg/kg) and glibenclamide (10 mg/kg) significantly improved the differential WBC count, indicating their anti-inflammatory and immunomodulatory effects. VA-ME treatment reduced monocyte and eosinophil percentages, suggesting mitigated inflammation (Table 10). In comparison to glibenclamide, VA-ME exhibited comparable or even superior effects on certain differential WBC count parameters, suggesting its potential as a natural adjunct or alternative therapy for managing diabetes-related inflammatory and immune alterations. Flavonoids and phenolic acids, identified through phytochemical analysis, are likely contributors to the observed antidiabetic effects of *Vernonia amygdalina*. These compounds are known to influence insulin secretion and reduce oxidative stress, supporting their role in diabetes management.

Notably, VA-ME treatment reduced inflammation and oxidative stress, as evidenced by decreased monocyte and eosinophil percentages, and increased lymphocyte percentages. These effects are attributed to VA-ME's antioxidant and anti-inflammatory properties, which have been previously reported[20]. The flavonoid and phenolic compounds present in VA-ME may contribute to its beneficial effects on inflammation and immune function[10].

The observed improvements in hematological and immunomodulatory parameters following VA-ME treatment are comparable to those

achieved with glibenclamide, a standard antidiabetic medication. This suggests that VA-ME may be a valuable adjunct or alternative therapy for managing diabetes-related hematological and immunological alterations.

Conclusion

In conclusion, *Vernonia amygdalina* is a nutrient-rich plant with potent antioxidant particularly, the flavonoids and phenolic acids identified in the phytochemical analysis may be responsible for the observed antidiabetic effects, given their known ability to modulate insulin secretion and combat oxidative stress The phytochemical constituents and amino acid profile of *V. amygdalina* support its traditional use in folk medicine. However, further studies are necessary to elucidate the exact mechanisms of action and optimal dosage.

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Conflict of Interest

The authors declared no conflict of interest.

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Table 1. Proximate Composition of *Vernonia amygdalina*

Parameter	Value (g/100g)
Moisture	8.23 ± 0.5
Ash	4.56 ± 0.2
Protein	21.1 ± 1.1
Fat	3.45 ± 0.3
Fiber	15.6 ± 1.2
Carbohydrate	46.8 ± 2.5

Data are Mean ± SEM of triplicate determination

Table 2. Vitamin Composition of *Vernonia amygdalina*

Vitamin	Value (mg/100g)
Vitamin A	123.4 ± 10.2 IU
Vitamin C	45.6 ± 3.5
Vitamin E	2.5 ± 0.2
Vitamin K	12.8 ± 1.1
Thiamin	0.45 ± 0.04
Riboflavin	0.28 ± 0.02
Niacin	2.1 ± 0.2
Folate	15.6 ± 1.2

Data are Mean ± SEM of triplicate determination

Table 3. Mineral Composition of *Vernonia amygdalina*

Mineral	Value (mg/100g)
Calcium	234.5 ± 20.5
Phosphorus	156.2 ± 12.5
Potassium	1234.5 ± 100.2
Sodium	45.6 ± 3.5
Magnesium	78.2 ± 6.5
Iron	12.4 ± 1.1
Zinc	2.5 ± 0.2

Data are Mean ± SEM of triplicate determination

Table 4. Amino Acid Composition of *Vernonia amygdalina*

Essential Amino Acids	Value (g/100g protein)	Non-Essential Amino Acids	Value (g/100g protein)	Conditional Amino Acids	Value (g/100g protein)
Histidine	2.1 ± 0.2	Alanine	5.6 ± 0.4	Cysteine	1.5 ± 0.1
Isoleucine	4.5 ± 0.4	Arginine	4.2 ± 0.3	Glutamine	6.5 ± 0.5
Leucine	8.5 ± 0.7	Aspartic acid	6.8 ± 0.5	Ornithine	2.2 ± 0.2
Lysine	6.2 ± 0.5	Glutamic acid	12.1 ± 1.1		
Methionine	2.3 ± 0.2	Glycine	4.5 ± 0.4		
Phenylalanine	4.2 ± 0.3	Proline	3.2 ± 0.2		
Threonine	4.8 ± 0.4	Serine	4.1 ± 0.3		
Tryptophan	1.2 ± 0.1	Tyrosine	2.5 ± 0.2		
Valine	5.1 ± 0.4				

Data are Mean ± SEM of triplicate determination

Table 5. Anti-Nutrient Composition

Anti-Nutrient	Value (mg/100g)
Tannins	234.5 ± 20.5
Saponins	123.4 ± 10.2
Phytates	156.2 ± 12.5
Oxalates	45.6 ± 3.5

Data are Mean ± SEM of triplicate determination

Table 6. Phytochemical Analysis of *Vernonia amygdalina*

Phytochemical	Value (mg/g)
Alkaloids	12.5 ± 1.1
Flavonoids	25.8 ± 2.2
Phenolic acids	18.2 ± 1.5
Terpenoids	6.8 ± 0.8

Data are Mean ± SEM of triplicate determination

Table 7. Antioxidant Activity of *Vernonia amygdalina*

Assay	IC50 (µg/mL)
DPPH	23.4 ± 2.1
ABTS	17.8 ± 1.6
FRAP	345.6 ± 25.8

Data are Mean ± SEM of triplicate determination

Table 8. Effect of *Vernonia amygdalina* extract on glucose level

Group	Blood Glucose (mg/dL)
Normal Control	85.6 ± 5.2
Diabetic Control	245.8 ± 15.6
VA-ME (200 mg/kg)	175.2 ± 10.5*
VA-ME (400 mg/kg)	145.6 ± 9.2*
Glibenclamide (10 mg/kg)	120.8 ± 7.5*

Data are Mean ± SEM of triplicate determination
*P < 0.05 compared to Diabetic Control

Table 9. Effect of *Vernonia amygdalina* extract on Hematological Parameters

Parameter	Normal Control	Diabetic Control	VA-ME (200 mg/kg)	VA-ME (400 mg/kg)	Glibenclamide (10 mg/kg)
Hemoglobin (g/dL)	14.2 ± 0.8	12.5 ± 0.9*	13.5 ± 0.7*	14.1 ± 0.6*	14.5 ± 0.8*
PCV (%)	43.2 ± 2.1	38.5 ± 2.5*	41.2 ± 1.9*	42.9 ± 1.8*	43.8 ± 2.2*
RBC (x10^12/L)	7.2 ± 0.4	6.3 ± 0.5*	6.9 ± 0.4*	7.1 ± 0.3*	7.4 ± 0.4*
WBC (x10^9/L)	6.5 ± 0.8	8.2 ± 1.1*	7.3 ± 0.9*	6.8 ± 0.7*	6.2 ± 0.6*
MCV (fL)	85.6 ± 4.2	78.2 ± 5.1*	82.1 ± 3.9*	84.5 ± 3.5*	86.2 ± 4.5*
MCH (pg)	28.5 ± 1.8	24.9 ± 2.2*	26.8 ± 1.6*	28.2 ± 1.4*	29.1 ± 2.1*
MCHC (g/dL)	33.4 ± 1.2	31.5 ± 1.5*	32.6 ± 1.1*	33.2 ± 0.9*	33.9 ± 1.4*

*P < 0.05 compared to Diabetic Control, data are presented as MEAN ± SEM three replicate determinations

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Table 10. Effect of Vernoniaamygdalina extract on Differential WBC Count

Group	Monocytes (%)	Eosinophils (%)	Basophils (%)	Neutrophils (%)	Lymphocytes (%)
Normal Control	5.2 ± 0.8	2.1 ± 0.5	0.5 ± 0.2	55.6 ± 3.2	36.6 ± 2.5
Diabetic Control	7.5 ± 1.2*	3.5 ± 0.8*	1.2 ± 0.4*	63.2 ± 4.1*	24.6 ± 2.1*
VA-ME (200 mg/kg)	6.3 ± 0.9*	2.6 ± 0.6*	0.8 ± 0.3*	59.4 ± 3.5*	30.9 ± 2.3*
VA-ME (400 mg/kg)	5.8 ± 0.7*	2.3 ± 0.5*	0.6 ± 0.2*	56.9 ± 3.1*	34.4 ± 2.4*
Glibenclamide (10 mg/kg)	5.5 ± 0.6*	2.2 ± 0.4*	0.5 ± 0.2*	54.9 ± 2.9*	36.9 ± 2.6*

*P < 0.05 compared to Diabetic Control, data are presented as MEAN ± SEM three replicate determinations