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Chemical composition, nutritional profile, and antioxidant potential of *Vernonia amygdalina*

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Abstract. The leaf of *Vernonia amygdalina* has been a known vegetarian food and medicinal plant used in Asia and Africa (West Africa) due to its observed pharmacological effects (antioxidant, antidiabetes, anti-inflammatory, anticancer, antimalaria, and among others). The present work investigated the leaf (proximate) and leaf extracts of two species of the plant (local and the hybrid species) and presented a comprehensive comparative qualitative and quantitative phytochemical screening, proximate (leaf) and vitamins analyses, antioxidant properties, and GC-MS and FT-IR characterization of the leaf extracts. The phytochemical results revealed the presence of tannin, alkaloid, flavonoid, saponin, glycoside, sterols and phenols in both local and hybrid species. The quantitative phytochemicals analysis revealed high content of alkaloids (51.3 mg/g), followed by flavonoids (44.3 mg/g) and saponins (34.7 mg/g). The antioxidant activity study showed values of 7.85±1.02 µM TE for CUPRA (Cupric Reducing Antioxidant Capacity) and 7.77±1.10 µmol Trolox/g for FRAP (Ferric Reducing Ability of Plasma). The GC-MS analysis revealed the presence of thirty-five bioactive compounds in both species, and the most applicable include 9, 12, 15-octadecatrien-1-ol, *n*-hexadecanoic acid, octadecatrienol acid, methyl palmitate, and phytol. The FT-IR results revealed the presence of functional groups consistent with O-H, C=O and C-H bonds in the methanol extracts of the plant. In the proximate study, the moisture content, ash content, crude fiber, fat content, crude protein and carbohydrate content were 33.15±0.03, 9.32±0.01, 11.83±0.02, 5.78±0.01, 9.13±0.01, and 30.79±0.02 %, in the local species, and 52.55±0.11, 6.76±0.03, 8.23±0.01, 6.54±0.21, 12.68±0.52, and 13.24±1.22 % in the hybrid species respectively. The vitamin analyses of the local species showed the highest amount of vitamin C while the hybrid species showed the highest amount of vitamin E. Antioxidant studies showed that the local species exhibited the highest antioxidant properties while the hybrid species showed highest antioxidant properties and the values for H_2O_2 scavenging ability of the species were 1.92±0.01 and 1.59±0.01 respectively. On a proximate basis, the hybrid showed a higher level of crude proteins and less carbohydrates.

***Keywords*:** *Vernonia amygdalina*; phytochemicals; vitamins; antioxidant; GC-MS and FT-IR characterization.

1. Introduction

Pro-oxidants and free radicals are produced in organisms as a result of exposure to various environmental and chemical variables associated with contemporary living, such as food, medications, cosmetics, smoke, radiation, and others. Oxidative stress is a state in which an excessive production of reactive oxygen species overwhelms an organism's natural antioxidant defenses, whether enzymatic, non-enzymatic, or dietary. In this state, cellular and extracellular macromolecules (proteins, lipids, and nucleic acids) can experience oxidative damage, leading to tissue injury [1]. Getting your hands on food that naturally contains phytochemicals and antioxidant activity, like different types of plants, fruits, and vegetables, is the best defense against tissue damage, unwanted changes, and health hazards of this kind. A compound known as an antioxidant has the ability to impede or stop biological macromolecules like proteins, DNA, and lipids from oxidizing unintentionally. Accordingly, fruits and vegetables have a preventive impact against chronic diseases because of their phytochemical composition, which is primarily phenolic, and consequent antioxidant activity [2]. *Vernonia* plants are a typical vegetable used in human nutrition. They are valued in particular for their recognized antibacterial and other biological properties, and they are utilized as food or a vegetable in dishes [1]. In traditional medicine, *Vernonia* species are frequently used to treat cardiovascular conditions and avoid the symptoms of infection. These species' phytochemical and antioxidant activities may account for some of the observed effects [3]. All around the world, bitter leaf, or Vernonia amygdalina, is used in daily cooking. Bitter leaf is the common name for *Vernonia amygdalina*; it is also referred to locally as "Shuwaka" in Hausa, "Ewuro" in Yoruba [4], and "Ityuna" in Tiv [5]. These two veggies have been used since ancient times. It has been suggested that eating bitter leaf reduces the risk of heart disease and malignancies of the stomach, colon, oesophagus, prostate, bladder, liver, lungs, breast, skin,

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and brain [6]. It has been demonstrated that bitter leaf extracts may scavenge radicals with exceptional efficiency and can prevent the growth of leukemia cells [7]. The molecules responsible for each of these biological have been reported in a review on antioxidant and bioadhesive properties of onions (*Allium* L., Alliaceae) processed under acidic conditions [8].

Chemical substances that are naturally present in plants are known as phytochemicals. They are in charge of the plant's color and organoleptic characteristics. The medicinal value of these plants lies in their phytochemical content [9]. Plants are endowed with various phytochemical molecules such as vitamins, terpenoids, phenolic, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other metabolites which are rich source of free radical scavengers [10].

2. Experimental

2.1. Materials and reagents

Vernonia amygdalina (local and hybrid species of bitter leaf) were bought from Wurukum, a local market in Makurdi, Benue State, Nigeria and taken to the laboratory, Department of Chemistry, Benue State University for preparation.

The chemicals used during the analysis are *n*-hexane (C_6H_{14}), ethyl acetate ($CH_3CO_2CH_2CH_3$), ferric chloride ($FeCl_3$), acetic anhydride ($(CH_3CO)_2O$), chloroform ($CHCl_3$), Fehling's reagents A and B, potassium dichromate ($K_2Cr_2O_7$), hydrogen peroxide (H_2O_2), hydrochloric acid (HCl), sodium hydroxide (NaOH), sodium thiosulphate ($Na_2S_2O_3{\cdot}5H_2O$), sulfuric acid (H_2SO_4), oxalic acid ($C_2H_2O_4$), potassium hydroxide (KOH), ascorbic acid ($C_6H_8O_6$) methanol (CH_3OH), ethanol (CH_3CH_2OH), trichloroacetic acid (CCl_3COOH), 2,6- dichlorophenol indophenol ($C_{12}H_7NCl_2O_2$). All the reagents were produced by JHD fine chemicals, China.

2.2. Sample preparation

The samples were washed with running tap water to remove surface contaminations and then air dried at room temperature for two weeks. The dried leafs were then pulverized into powder using mortar and pestle and stored in air-tight containers for further analyses.

2.3. Extraction of phytoconstituents

The cold maceration extraction method was employed to extract the chemical constituent in the *Vernonia amygdalina* leaves. The solvents used in this extraction were *n*-hexane and methanol. Exactly 100 g of the powdered samples were soaked with 350 mL of *n*-hexane separately and left for 72 hours in a 500 mL baker in closed container and same for methanol. The *Vernonia amygdalina* leaves extracts were evaporated by using the rotary evaporator at 45 ºC. The process was continued until no notable changes were observed.

2.5. Phytochemical analysis

Phytochemical screening for qualitative analysis was done according to the method described by Moussaoui and Alaoui [11]. The quantitative analysis was done according to the methods described in the literature [12-14]. The total nitrogen content was determined by the Kjeldhal method and converted to crude protein by using a standard conversion factor (6.25) [15].

GC-MS analysis of bioactive compounds from the extract was done using Agilent Technologies GC equipment. Specifically, the GC-7890A/MS-5975C model (Agilent Technologies, Santa Clara, CA, USA) was used with an HP-5MS column (30 m length, 250 µm diameter, 0.25 µm film thickness). The electron ionization method used in GC-MS spectroscopic detection was 70 eV electrons. This study used 99.995% pure helium gas as a carrier. Carrier gas flowed at 1 mL/min. The temperature was first set between 50 and 150 °C and increased by 3 °C every minute. This temperature was maintained for 10 min. The temperature rose 10 °C every minute to 300 °C. An adequate solvent-diluted 1 µL of the produced 1% extracts was split less administered into the system. Each extract's chemical component percent was calculated using the chromatogram peak area.

The identification of phytoconstituents derived from the extract was performed through the analysis of their gas chromatography retention time on an HP-5MS column, as well as by comparing their spectra with computer software data of established standards from GC–MS systems, namely Replib and Mainlab data.

Furthermore, the FTIR analysis was done with the Buck Scientific M530 USA. A deuterated triglycine sulfate detector and potassium bromide beam splitter were used. Gramme A1 programme collected and altered spectra. A 1.0 g sample was well mixed with 0.5 ml of nujol and put over the KBr salt pellet. Fourier Transform Infrared (FTIR) spectra were taken from 4,000 to 400 cm^{-1} during measurement. Co-addition with 32 scans and 4 cm^{-1} resolution merged these spectra. Transmitter values were used to display FTIR spectra.

Proximate analysis was done according to the method described by AOCA [15], and was performed only on the plant leaf.

The vitamins A, C and E in the sample were determined using the spectrophotometric methods described by Achikanu et al. [16]. The absorbance was measured at 620 nm (for the determination of vitamin A) and 520 nm (vitamin C and E).

The antioxidant activity was determined using FRAP (Ferric reducing ability of plasma), CUPRAC (Cupric Reducing Antioxidant Capacity) and hydrogen peroxide (H_2O_2) scavenging methods according to the methods described by Bhatti et al. [17].

3. Results and discussion

The qualitative phytochemical screening, proximate composition, vitamin content and antioxidant properties of two species of *Vernonia amygdalina* extracts are presented in Tables 1-5.

Table 1. Qualitative phytochemical screening of *Vernonia amygdalina* extracts

Phytochemicals	Bitter leaf extract species	
	Improved	Local species
Alkaloids	+++	+++

Phytochemicals	Bitter leaf extract species	
	Improved	Local species
Tannins	+++	+++
Flavonoids	+++	+++
Saponins	++	++
Glycosides	+++	+++
Sterols	+++	+++
Phenols	++	++

Key: + = present; - = absent; solvent = *n*-hexane

Most phytochemicals serve as natural antibiotics, which assist the body in fighting microbial invasion and infections [18]. In this research, all phytochemicals tested were found present in both local and the hybrid species of *Vernonia amygdalina* (Table 1). The result is similar to the report by Achinewhu et al., who confirmed the presence of tannins, alkaloids, saponins, flavonoids, sterols phenols, and terpenoids in local *Vernonia amygdalina* [19].

Table 2. Quantitative phytochemical results of methanolic extract of bitter leaf

Composition	Bitter leaf extract species (mg/g)	
	Improved	Local species
Alkaloids	51.30±0.20	47.10±0.10
Tannins	8.70±0.01	5.60±0.02
Flavonoids	41.30±0.30	44.30±0.20
Saponins	25.10±0.10	34.70±0.20
Terponoids	5.70±0.01	8.60±0.04
Phenols	5.90±0.02	5.80±0.05

Table 2 gives information on the chemical composition of the methanol extract of bitter leaf and revealed the presence and amount of phytochemicals that have been documented to have antioxidant and other activities.

Table 3. Proximate composition of *Vernonia amygdalina* extracts

Parameters	Bitter leaf extract species	
	Improved	Local species
Moisture content (%)	52.55±0.11	33.15±0.03
Ash content (%)	6.76±0.03	9.32±0.01
Crude fiber (%)	8.23±0.01	11.83±0.02
Crude protein (%)	12.68±0.52	9.13±0.01
Carbohydrate (%)	13.24±1.22	30.79±0.02

The contents of moisture, ash, fiber, fat, protein, and carbohydrates were assessed in the proximate analysis (Table 3). The sample's moisture content controls both its water and, indirectly, its dry matter contents. Additionally, it gauges how stable samples are throughout storage [20]. Substances with a moisture content of more than 14% are less storable due to their susceptibility to microbial development [20], indicating that the product's shelf life will be brief. Foods' amounts of ash provide information about their mineral composition. The hybrid species' 6.76±0.03 % had a lower ash content value than the indigenous species' 9.32±0.01%. This conclusion is similar to that of Aremu et al. [21] and Olaofe et al. [22]. The crude fiber content of the hybrid species was 8.23±0.01%, while the local specie's was 11.83±0.02%. This indicates that a reduction in crude fiber may result from a gene mutation in this plant. Both figures, however, are greater than the 3.8% crude fiber content for spinach that has been reported [21]. Research indicates that dietary fiber's indigestibility in the small intestine contributes to a number of health benefits [22]. When compared to other leaves, including those from cactus plants (47.9 – 51.1 %), *Vernonia amygdalina* does not qualify as an oil-rich plant because its crude fat content was somewhat greater in the hybrid species (6.54±0.21 %) than in the indigenous species (5.78±0.01 %) [22]. In addition, they are high in energy and help absorb fat-soluble vitamins, fats are vital in diets. The hybrid *Vernonia amygdalina* has a crude protein value of 12.68±0.52%, whereas the native species had a crude protein content of 9.13±0.01%. Studies have shown that the crude protein content of *Vernonia amygdalina* can be increased via gene mutation. When processed, it could be utilized as a supplement or alternative source of protein in the diet, particularly in a country like Nigeria where the bulk of the population subsists on cereals and starchy foods.

Compared to the hybrid species (13.24±1.22%), the native species had a greater carbohydrate content (30.79±0.02%). The amount of carbohydrates in *Vernonia amygdalina* suggested that it was a rich source of energy, capable of meeting both children's and adults' bodies' daily energy needs.

Table 4. Vitamin contents of *Vernonia amygdalina* extracts (mg/100 g)

Vitamins	Bitter leaf extract species	
	Improved	Local species
A	1.61±0.01	2.25±0.03
C	4.97±0.11	4.80±0.10
E	16.95±2.13	4.06±0.11

The findings of measuring the species' content of vitamins A, C, and E showed that the hybrid species had the largest amount of vitamin E and the local species had the highest amount of vitamin C (Table 4). According to Bhatti et al. [17], the local *Vernonia amygdalina* contained 6.21% vitamin A, 4.83% vitamin C, and 3.74% vitamin E. The discrepancy in the results on Table 4 may be caused by variations in the analytic techniques or botanical provenance.

Table 5. Antioxidant properties of *Vernonia amygdalina* extracts

Antioxidant parameters	Bitter leaf extract species	
	Improved	Local species
FRAP (µmol Trolox/g)	7.77±1.10	1.27±0.01
CUPRAC (µM TE)	4.85±0.10	7.85±1.02
H_2O_2 (100 µg/mL)	1.59±0.01	1.92±0.01

The antioxidant characteristics of the samples were displayed in Table 5 in relation to H_2O_2, ferric reducing antioxidant power (FRAP), and cupric reducing antioxidant capacity (CUPRA). Evidently, the hybrid species demonstrated the strongest antioxidant capabilities towards FRAP (7.77±1.10), whereas the local species demonstrated the highest antioxidant properties towards CUPRAC (7.85±1.02). In general, the test molecules were ranked in the following order of

antioxidant activity in all samples: FRAP > CUPRAC > H_2O_2 in hybrid species, and CUPRAC > FRAP > H_2O_2 in the local *Vernonia amygdalina*. This species' strong antioxidant capabilities against the test compounds are a result of the phytoconstituents found in *Vernonia amygdalina*.

Figures 1 and 2 show the various peaks as observed by the gas chromatogram and the results gave 11 peaks that correspond the following bioactive components, which included squalene, vitamin E, phytol, 9,12,15-octadecatrienoic acid, methyl ester (Z, Z, Z), 9,12,15-octadecatrienoic acid (Z, Z, Z), neophytadiene, *n*-hexadecanoic acid, *n*-hexadecanoic acid, and methyl ester amongst others.

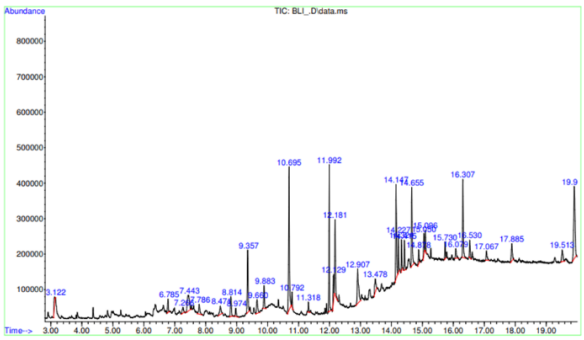

Figure 1. GC-MS chromatogram of methanolic extract of bitter leaf local specie.

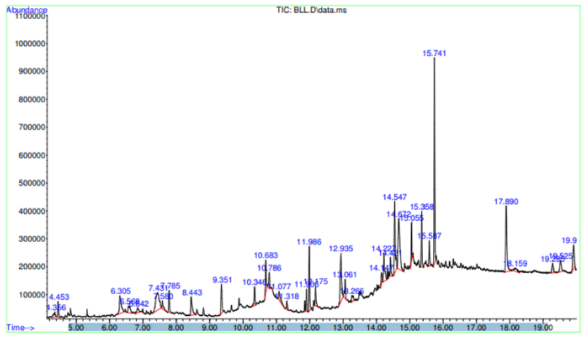

Figure 2. GC-MS chromatogram of methanolic extract of bitter leaf improved specie

The GC-MS analysis showed the presence of 11 phytochemical constituents which may contribute to the plant antimicrobial, antioxidant, anticancer, hypercholesterolemic, anti-inflammatory, and other activities. Hence, the presence of these phytochemicals may be responsible for their therapeutic effects.

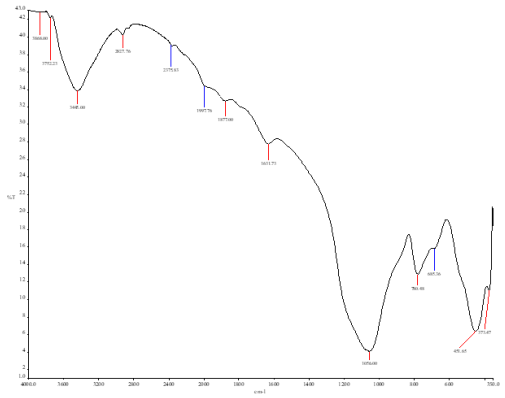

Figure 3. FTIR spectrum of methanolic extract of bitter leaf local specie

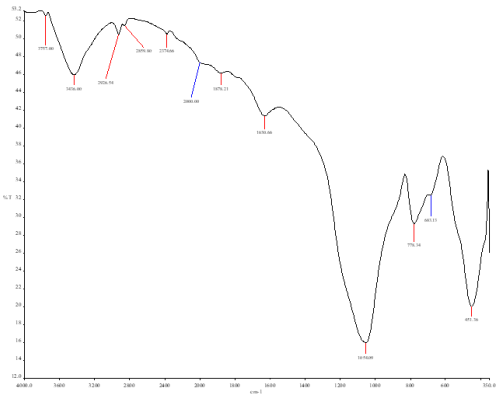

Figure 4. FTIR spectrum of methanolic extract of bitter leaf improved specie

From the spectra in Figures 3 and 4, it is observed that the strong absorption bands around 3445 cm^{-1} and 3436 cm^{-1} correspond to O-H stretching and are broad due to the intermolecular hydrogen bonds. The absorption bands at 2859 cm^{-1} and 2927 cm^{-1} can be attributed to C-H symmetric and asymmetric stretching respectively as in methylene (CH_2). The absorption band at 2375 cm^{-1} can be attributed to a carbon-carbon triple bond (C≡C), while absorption band at 1878 cm^{-1} and 1630 cm^{-1} can be attributed to carbonyl compounds but not aldehyde due to the absence of bands of 2800 cm^{-1} and 2700 cm^{-1}. The strong band at 1056 cm^{-1} corresponds to the C-O stretching. The analyzed sample may contain acids, amide and alcohol groups. The studied materials have ester and ketone related components, and these information are in agreement with the organic compounds found in the GC-MS analysis.

4. Conclusions

The findings of this study have demonstrated that the phytochemicals tannins, alkaloids, flavonoids, saponins, phenols, glycosides, and steroids are present in the leaves of both native and hybrid species of *Vernonia amygdalina* and are what give the plant its antioxidant properties. The hybrid species' high vitamin E content may also be the cause of its strong antioxidant action. The bitter leaf also has shown to have various secondary metabolites which possess many pharmacological properties of which antioxidant activity is one. The GC-MS analysis showed the presence of 11 phytochemical constituents that may contribute to the antimicrobial, antioxidant, anticancer, hypercholesterolemic, anti-inflammatory, and other activities of the plant. The characterization using FTIR indicated the presence of functional groups such as O-H and C-O.

Conflict of interest

The authors declare no conflict of interest.

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