STUDY ON NUTRITIONAL PROPERRTIES AND ANTIOXIDANT ACTIVITY OF VERNONIA AMYGDALINA DEL. LEAF.

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ABSTRACT

The current study focuses on investigating the nutritional properties and antioxidant activities of *Vernonia amygdalina leafy vegetable commonly used by people of North Central and Southern parts of Nigeria. The plants have been used as vegetable different parts of Nigeria due to its nutritional values and as herbs in the treatment of various ailments. The sample of the plant was harvested in Lokoja, Kogi State, North Central Nigeria.* The proximate, minerals and anti-nutrients composition as well as antioxidant activity of *V. amygdalina leaves* were evaluated using standard procedures. Results of proximate analysis showed a composition of **8.8**% moisture, **7.05**% ash, **19.01**% crude protein, **8.13**% crude fibre, **3.67**% crude lipid, **62.14**% available carbohydrate and **353.95** kcal/g of energy value. The mineral elements in the leaf are sodium (**1524.08**mg/100g), potassium (**2025.48**mg/100g), calcium (**428.29**mg/100g), phosphorus (**6.49**mg/100 g) and magnesium (**564.74**mg/100g). The mineral ratios (Na/K and Ca/P) were **0.75 and 66** respectively. The anti-nutritional factors present in the leaves include phytate (**2.60**mg/100ml), oxalate (**3.28**mg/100ml) and tannins (**0.63**mg/100ml). The antioxidant activity was determined and the IC₅₀ values for the sample and the standard were 29.33 and 33.22 respectively. Based on the results obtained, the leaves of this plant could serve as a cheap source of nutrients as well as a supplementary source of natural antioxidants needed by the body.

Keywords: Antioxidants, Nutrients, Minerals, Vernonia amygdalina

1.0 INTRODUCTION

Vernonia amygdalina is an ever-green shrub belonging to the family of Asteraceae. It grows predominantly in tropical Africa particularly in Nigeria, Cameroon and Zimbabwe . It is also found in places like tropical America, Madagasca and Asia, where it occurs as herb or shrub (Atangwho *et al.*, 2009). In Nigeria, the plant is called "shuwaka" among the Hausas, "Ewuro" in Yoruba and "Olugbu" in Igbo . Due to the **characteristic astringent bitter taste of** *V. amygdalina*, it is *commonly known as bitter leaf*.

V. amygdalina is a popular leafy vegetable consumed by people of North Central and southern parts of Nigeria, where it is used as spice in the preparation of popular "bitter-leaf soup **. It has been** widely studied by researchers due its dual properties of food and medicine.

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The leaf has been reported to contain appreciable amount of both macro and micro nutrients needed by the body for proper physiological activities. The plant rich in essential amino acids which can make it a potential source of protein. It contains mineral elements such as potassium, sodium, calcium, magnesium, zinc, manganese and iron which are needed by the body in different concentration for healthy being .

Various bioactive phytochemicals were detected in extracts of V. amygdalina; and these may be responsible for the reported medicinal properties of the plant

The aqueous extracts of V. amygdalina has been in use for decades by traditional health workers in Africa for the treatment for varieties of ailments ranging from emesis, nausea, diabetes, loss of appetite, dysentery and other gastrointestinal tract problems to sexually transmitted diseases and diabetes mellitus among others

The plant was reported to show antihelmitic, antimalarial antitumourigenic, hypoglycaemic and hypolipidaemic activities. It was also reported to contain essential vitamins such as vitamins A, E and C which are believed to possess antioxidant activities. Various studies were conducted in other locations to evaluate the nutritional and antioxidant properties of the plant.

The current study was carried out with a view of establishing the possible variations in the nutritional and antioxidant properties of V. amygdalina harvested in Lokoja Kogi State, relative to those earlier reported. Variations that could be due to

soil nutrients and environmental factors that may have effects on the nutrients availabilities and phytochemical constituents of the plants

2.0 MATERIALS AND METHODS 2.1 Sampling and sample Preparation

Freshly harvested leaves of *Vernonia amygdalina* were obtained from Lokongoma local market in Lokoja, Kogi State, Nigeria. The leaves were carefully removed from the stalk and air dried for seven days. The dried leaves were pulverized into fine powder using laboratory mortar and pestle. The powdered sample was stored in an air tight container prior analysis.

2.2 Extraction of Plant Material for Antioxidant Activity Determination

50 g of the powdered leaves were soaked in 500 ml of methanol with continuous agitation for 48 hours. The mixture was filtered with a Whatman No. 1 Filter paper. The filtrate was concentrated to dryness at 40°C using a rotary evaporator.

2.3 Proximate Analysis

2.3.1. Determination of moisture (AOAC, 2000).

Moisture was determined by oven drying method. 2 g of powdered sample was accurately weighed in clean, dried crucible (W). The crucible was allowed in an oven at 100-105°C for 6-12 h until a constant weight was obtained. Then the crucible was placed in the desiccator for 30 min to cool. After cooling it was weighed again (W). The percent moisture was calculated by following $%Moistur = \frac{W1 - W2}{Weight of the sample} \times 100$

Where:

. ...

 $W_1 =$ Initial weight of crucible + Sample $W_2 =$ Final weight of crucible + Sample.

2.3.2. Determination of ash content (AOAC, 2000).

Clean and empty crucible was placed in a muffle furnace at 600°C for an hour, cooled in desiccator and then weight of empty crucible was noted (W_1) . 2 g of powdered sample was taken in crucible (W_2) .

The crucible was placed in muffle furnace at 550°C for 2-4 h. The appearances of gray white ash indicate complete oxidation of all organic matter in the sample. The crucible was removed from the furnace, cooled and weighed (W₃). Percent ash was calculated as follows:

 $%Ash = \frac{W3 - W1}{weight of the sample} \times 100$

2.3.3 Determination of lipid, protein, carbohydrate and energy contents (AOAC, 2000).

To determine the total lipid content, the sample was extracted with petroleum ether (60-80 °C) in a Soxhlet apparatus for about 6-8 h. The residual solvent was allowed to evaporate in a preweighed beaker. increase in weight of beaker gave the total l lipid content of the sample.

Nitrogen content in the sample was estimated by using micro Kjeldahl method and crude protein was calculated by multiplying the evaluated nitrogen by 6.25.

Available carbohydrates were determined by difference : 100-(percentage of ash +

percentage of total lipid + percentage of
protein + percentage of crude fiber).
Energy value of the leaf sample was
estimated as ;
Energy (calorific) value (kcal/100g) =

Energy (calorific) value (kcal/100g) = (Crude lipid x 8) + (Crude protein x 4) + (Carbohydrate x 4)

2.3 Mineral Analysis

2.3.1 Sample Digestion

The sample (0.5 g) was put into Kjeldahl digestion flask to which 24**ml** of a mixture of concentrated nitric acid (HNO₃), conc. H₂SO₄ and 60% HClO₄ (9:2:1v/v) was added. The flask was kept overnight. The flask was put on a heating block and digested to a clear solution, cooled and the content filtered into a 50 **ml** volumetric flask. The solution was then diluted to volume with distilled water. Blank solution

was prepared in similar manner without sample being added. The atomic absorption spectrophotometer (AAS) was used for the analyses of Mg, Ca and P while the flame photometer was used in the analyses of K and Na.

2.4 Estimation Radical Scavenging Activity

The in vitro free radical scavenging capacity of the methanol leaf extract a g a i n s t D P P H (1,1-diphenyl-2-picrylhydrazyl) free radical was determined by method of . Ascorbic acid was used as standard reference compounds. The scavenging activities of the extract and the standard were calculated as follows;

% SA=(A_{blank} - $A_{extract}$)/ A_{blank} x 100

Where A_{blank} is the absorption of the blank sample and $A_{extract}$ is the absorption

of the extract.

Inhibition concentration, IC_{50} (concentration of extract or standard required to scavenge 50% of DPPH radicals), were determined from the linear regression curve.

2.5 Statistical Analysis

All analyses were carried out in triplicates and Analysis of Variance (ANOVA) was conducted to determine significant differences.

3.0 RESULTS AND DISCUSSION

Table 1:Proximate Composition of V.amygdalina leaf.

Parameter	Concentration (% DW)
Moisture content	8.85±0.14
Ash content	7.05±0.14
Crude protein	19.01±0.09
Crude lipid	3.67±0.06
Crude fibre	8.13±0.15
Available	62.14±0.39
carbohydrate	
Calorific value	353.95±1.03
(Kcal/100g)	

Values are mean \pm SD of triplicate determinations The results of proximate analysis of the *Vernonia amygdalina* leaf was presented in Table 1. The moisture content obtained in this study for the *V. amygdalina leaves* was 8.85 \pm 0.145 \pm 0.14%. This value is close to the values reported for some common vegetables e.g. Amaranthus hybridus (8.35 \pm 0.0%5) Curcubita pepo (8.55 \pm 0.01%) and Gnetum africana (7.60 \pm 0.01%) reported by . The value is very low compared to value 91.05±1.41% obtained for *Lepidium sativum* leaves . Moisture content of food material is a good parameter to measure the susceptibility to microbial attack. High moisture content indicates that the food is prone to spoilage.

The total ash content of *the sample* was 7.05±0.14%. The value was higher compared

to values 1.1 ± 0.15 % reported in Spinacia oleracea, 0.8 ± 0.01 % in Lacluca Sativum and 0.6 ± 0.01 % in Brassica oleracea capitata respectively. The value is lower than 22.18 ± 0.5 % in launaea taraxacifolia leaves . Ash content is the index of total mineral elements present in plants materials. The value obtained indicates that the plant may be considered to bear good source of minerals needed by the body for proper physiological activity.

The crude protein content of V. amygdalina leaf sample was found to be 19.01±0.09%. This value was higher compared to values 1.53±0.00% in Ficus thonningii, 0.85±0.14% in Annona senegalesis and 0.28±0.39% in Emilia coccinea . Proteins are essential component of diet needed for survival of animals and humans. The basic function of protein in nutrition is to supply the body with adequate amounts of required amino acids. It is also necessary for building the structural components of human body, such as muscles and organs.

The amount of crude fibre obtained in the sample was $8.13\pm0.15\%$. The value is very low compared to the values 19.53 ± 0.06 in Allium cepa, 24.08 ± 0.0 in Spinacia

oleraceae and 23.30 ± 0.01 in Coriandrum sativum , and higher than the values 0.7 ± 0.06 in **Spinacea** oleracea, 1.4 ± 0.07 in Momordica charantia and 0.4 ± 0.05 Portulaca oleracea. It was reported that dietary *fibre* helps in reducing the risk of coronary heart diseases, breast cancer and hypertention . reported that consumption of foods rich in fibre may contribute to reduction in the incidence of certain diseases like high blood pressure, heart disease, coronary colon cancer, diabetes, obesity and various digestive disorders.

The crude lipid content of the sample was 3.67±0.06. This value is higher than 2.07±0.23 in *Abelmoschus esculentus* and lower than 9.32±0.6 in *Moringa olifera*. The value is low and this in agreement with general observation that leafy vegetables are poor sources of lipids.

Generally as could be seeing from the values obtained fromproximate analysis of the leaf of sample vary from one location to another. Difference in soil nutrients and environmental factors may be responsible for the variations.

It is believed that consumption of food rich in vegetables may play significant role in avoiding obesity due to the significantly low lipid content of leafy vegetable.

Available carbohydrate obtained from the sample was 62.14±0.39%, this value is higher compared to the values 12.26±0.12% and 14.44±1.6% in *Musanga cercropioides* and *Maesobotrya barteri* respectively . If properly utilized, the plant can serve as a good source of energy and provide fuel to the body for physical performance such as breathing, maintaining body temperature and contraction and relaxation of muscle .

Table	2:	Minerals	content	of	<i>V</i> .
amygdalina leaf					

Elements	Concentratio n (mg/100g)	Recommended Daily Allowance (RDA) (mg/day)	
		Adult	Infant and Children
Potassium	2025.48±0.16	2000	1600
Sodium	1524.08±0.89	500	400
Calcium	428.29±3.29	1000	400-600
Magnesium	564.74±0.89	350	60
Phosphorus	$6.49 \pm 0.0.04$	800	300-500
Na/K	0.75		
Ca/P	66		

The leaf sample of *A. amygdalina* was analyzed to determine the concentrations of different mineral elements present, the results of which is shown in Table 2. The level of potassium was highest (2025.48 mg/100g) and phosphorus appeared to be the lowest (6.49 mg/100g). The high level of potassium in the sample is in agreement with observation that, plant based foods are usually high in potassium .

Sodium and potassium play important roles in maintaining normal physiological function of the body. They are important intracellular and extracellular cations respectively, which are involved in the regulation of plasma volume, acid-base balance, nerve and muscle contraction ;. Na/K ratio of the sample was determined and found to be 0.75. This is due to low sodium and high potassium content of the sample. With the value of sodiumpotassium ratio less than one, the sample could contribute positively in the management of high blood pressure

The calcium content of the sample was found to be 428.29 ± 3.29 mg/100g, which is within the range of values (91.13 – 873.33 mg/100ml) for common leafy vegetables in south eastern Nigeria . Calcium plays an important role in the body which include; formation and sustenance of strong bones and teeth at both early and later life , regulation of nerve and muscle function and in blood clotting, muscle contraction and for the activity of certain enzymes metabolic processes.

Ca/P ratio of the leaf was high (66), this is due to the lowest level of Phosphorus in the leaves. However, this may be of advantage, because a diet is considered good if the calcium to phosphorus ratio (Ca/P) is > 1 and as poor if < 0.5. It was also reported that high intakes of diet of high phosphorus and low calcium content may be associated with a lower calcium to phosphorus ratio (Ca:P) which potentially has adverse health effects, including arterial calcification, bone loss, and death .The leaves of V. amygdalina shows a high amount of magnesium o f 564.74±0.89mg/100mg. The RDA value for magnesium for adult and lactating woman is 350 mg/day. This implies that,

the leaves could supply adequate magnesium required by the body if properly utilized. Magnesium plays a key roles in the metabolism of calcium in bones and in the control and prevention of heart diseases

Table 3: Antinutritional Content of V.amygdalina

Antinutrients	Concentration (mg/100g)
Oxalate	3.28±0.03
Phytate	2.60±0.03
Tannins	0.63±0.02

Values are mean ± SD of triplicates determinations.

V. amygdalina leaves where analyzed to quantify various antinutrional factors and the result was presented in Table 3. The antinutrients analyzed were oxalate, phyatate, tanins and hydrogen cyanide.

The level of tannins in the leaves was found to be 0.63±0.02mg/100g. The value is lower than what was reported in some underutilized green leafy vegetables of Sonitpur district of Assam, India . The adverse nutritional effects of tannins include their ability to inhibit the activities of digestive enzymes and formation of tannin-protein complexes which may decrease protein digestibility . From medicinal point of view, tannins, as phenolic compounds, were reported to possess various medicinal activities such as anti-inflammatory, anti-hemorrhagic and antiseptic properties

The phytate content of the leaves was 2.60±0,03mg/100g. This value is lower than 10.95±2.66mg/100g obtained in

Lepidium sativum leaves . Phytate binds with positively charged food components, such as proteins, carbohydrates, minerals and mineral elements to form binary complexes such as protein-phytate complexes, carbohydrate-phytate complexes via hydrogen bonds and mineral-phytate complexes which make them less bioavailable in the body .

Table	4:	Antioxidant	activity	of	<i>V</i> .
amygdalina leaf					

Concentration	Inh	ibition (%)
(µg/mL)	Extract	Standard
		Ascorbic Acid
100	27.80±0.20	31.37±0.20
200	40.60±0.40	46.83±0.20
400	55.71±0.35	59.63±0.20
600	67.70±0.20	75.43±0.35
800	70.13±0.20	83.97±0.20
1000	83.85±0.20	92.54±0.18
IC_{50}	29.33	33.22

Values are mean ± SD of triplicate determinations

Table 4 presents the result of the antioxidant activity of the leaf sample. The antioxidant activity of the methanol extract of the *V. amygdalina leaf* was studied using the DPPH scavenging assay. The scavenging power of the extract was determined and compared with the reference standard antioxidant ascorbic acid. A free radical accepts an electron donated by an antioxidant compound and becomes paired off. The DPPH assay provides information on the reactivity of the antioxidant compounds with a stable

free radical. DPPH gives a strong absorption band at 517nm in visible region. On acceptance of an electron from a free radical scavenger, the absorption reduces and the DPPH solution is decolourized as the colour changes from deep violet to light yellow . The methanol extract of the V. amygdalina demonstrated a strong free radical scavenging activity that is closely similar to the standard ascorbic acid at all concentrations tested. The inhibition concentration (IC $_{50}$ values) of the extract and standard were evaluated and found to be 29.33 and 33.22 respectively. The high inhibition was observed at concentration of 1000µg/ml leave extract.

4.0 CONCLUSION

The current study has revealed the nutritional composition and mineral contents of V. amygdalina leaves from Lokongoma local market in Lokoja, Kogi State, Nigeria. The values obtained from proximate analyses of the leaf of sample vary from one location to another. Difference in soil nutrients and environmental factors may be responsible for the variations. However, result showed that the leaves of the plant are rich in proximate and minerals compositions. Antioxidant analysis on the methanol extract of the sample showed that the leaves possess a strong scavenging activity against DPPH free radical. It can therefore be concluded that the leaves of V. amygdalina can serve as a source of macro and micro nutrients needed by the body for proper functioning and as a cheap source of natural antioxidant needed to scavenge the

free radical species thereby preventing diseases that are caused as a result of oxidative stress.

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