



Research Article

Proximate Analysis, Mineral Content, Amino Acid Composition and Functional Properties of *Vernonia amygdalina* Vegetable Leaf Protein Concentrates

Sodamade A.

Department of Chemistry, Emmanuel Alayande College Ofof Education. P.M.B 1010, Oyo.

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ABSTRACT

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***Corresponding Author**

Sodamade A.

E-mail: abbey4uselect@yahoo.com

or abbey01@fastmail.net or

abbeyelectrochemical@hotmail.com

Freshly harvested *Vernonia amygdalina* green vegetable was authenticated and processed for its vegetable leaf protein concentrates with a view to evaluate its proximate constituents, amino acid content and mineral composition. Proximate analysis was determined using standard analytical technique. The nutrient composition of the protein concentrates revealed the moisture content (66.45 ± 0.07), Crude fat (9.20 ± 0.85), Crude fibre (10.46 ± 1.05), Crude protein (44.28 ± 2.43), ash (12.48 ± 0.24) and Carbohydrate (23.58 ± 3.4). The mineral content of the sample indicated that Ca, Mg, K, and Na are the most abundant minerals with the following values Na; 57.5 ± 0.34 , Ca; 151.6 ± 1.40 , K; 61.5 ± 0.38 , Mg; 96.5 ± 0.96 , other minerals that were present in the sample in trace concentration are Fe (2.3 ± 0.42), Zn (1.11 ± 0.11), Mn (0.1 ± 0.14), Cu (0.1 ± 0.73), Pb (0.1 ± 0.63), while Selenium were not detected in the sample indicating that the leaf concentrate is fit for dietary consumption. The amino acid profile reveals favourable nutritional balance with the presence of essential and non-essential amino acid except that tryptophan which was believed to be predominant in animal protein was not detected.

Keywords:

Leaf protein, leaf concentrate,

Vernonia amygdalina

INTRODUCTION

Several vegetable species are found in tropical and subtropical regions of the world where they are used partly as condiments or spices in human diets or as a supplementary feeds to livestock (Aletor and Adeogun, 1995). Vegetables have been discovered to have almost all of the mineral and organic nutrients established as essential for human nutrition and this nutritional information are used increasingly by public agencies and agricultural industries to promote fresh product. People are looking for variety in their diets and are aware of the health benefits of fresh fruits and vegetables.

Vernonia amygdalina also known as Bitter leaf is a member of the squash family of plants. They are consumed as cooked complements to major staple foods such as cassava, pounded yam, guinea corn, maize, millet, rice and plantains. It is also considered for its high medicinal value as the juice extracted from the leaves are wholly applied to fresh wound or cuts in some rural community (Adanlawo and Dairo, 2006).

Furthermore, it is well known that proteins are of great importance to health, and are often deficient in the diets of people in developing countries, especially those in the vulnerable groups, such as nursing mothers, expectant mothers, weanlings and pre-school children (Fasuyi and Aletor, 2005).

However, since the last world war, emphasis has been placed on the need to increase the dietary protein, particularly by the use of locally grown vegetables protein as well as the increased use of edible fish, meal as a result of the realization that the diet in underdeveloped countries is chronically low in protein leading to malnutrition and wide spread deficiency diseases. As a result, nutritionist are researching on suitability of vegetables that has promising values as a means of replacing proteins from animal sources which are very expensive and economically unviable (Oke, 1973).

However, extraction and analyses of some common leaf vegetable protein concentrates are common in literature using various techniques but the only predominant information about *Vernonia amygdalina* in literature are its medicinal value, herbal value and nutritional value. There has been no information about its leaf protein concentrates, therefore the purpose of the present investigation is to determine the proximate composition, amino acid content and Mineral composition of *Vernonia amygdalina*'s leaf protein concentrates.

MATERIAL AND METHODS

A healthy and improved authenticated breed of *Vernonia amygdalina* used for this study was obtained from vegetable garden located near Erelu dam of Oyo town and authenticated. Analar grades of petroleum ether,

HCl, copper, sulphate, selenium dioxide, sulphuric acid, anhydrous sulphate, etc were obtained from chemical store.

Preparation of Samples

The roots of vegetable were cut off; the vegetable was washed with distilled water and pulped by passing it through the locally produced mincer (technically referred to as cell rupture). The pulp was collected and strained through a cotton cloth. The green juice obtained above (pulp) was heated between 85° to 90°C by steam injection, which resulted in coagulation of all the protein present within the pulp. The coagulum was then centrifuged from the rest of the solution which were subjected to experimental investigation.

Proximate Analysis

Proximate analysis was carried out by the various method described by AOAC (1990).

Determination of Amino Acid

1g of the defatted coagulated leaf concentrate of *Vernonia amygdalina* was weighed into ampoule. 7ml of 6N HCl was added after nitrogen has been passed into the ampoule to remove any trapped oxygen and to avoid oxidation of some amino acid during hydrolysis. The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at 105°C for 22 hours. The ampoule was allowed to cool before broken opening the tip and the content was filtered to remove the humans. The filtrate was then evaporated to dryness at 40°C under vacuum in a rotary evaporator. The residue was dissolve with 5ml of acetate buffer pH 5.0, stored in plastic specimen bottles, and kept in deep freezer (Sparkman et al., 1958) before it was then loaded into amino acid analyzer.

Mineral Analysis: Each of the samples (1.0g) was weighed and subjected to dry ashing in a well-cleaned porcelain crucible at 550°C in a muffle furnace. The resultant ash was dissolved in 5.0ml of HNO₃/HCl/H₂O (1:2:3) and heated gently on a heating mantle until brown fumes disappeared. 5.0ml of distilled water was added to each of the sample in arable and heated until a colourless solution was obtained. The mineral solution was filtered into a 100.0ml volumetric flask through filter paper, and the volume was made to the mark with distilled water. The solution was analyzed in triplicate for its elemental composition using perking Elmer 403 model atomic absorption spectrophotometer.

RESULTS AND DISCUSSION

Table 1: The Proximate Composition (mg/100g) of *Vernonia amygdalina*'s Leaf Protein Concentrates

PROXIMATE COMPOSITION	VALUES (mg/100g)
Moisture	66.45±0.07
Fat	9.20±0.85
Fibre	10.46±1.05
Protein	44.28±2.43
Ash	12.48±0.24
Carbohydrate	23.58±3.4

±; mean triplicate determination

The proximate constituents obtained from the sample were presented in the Table 1 above.

The value moisture content is 66.45±0.07; moisture in food determines the keeping quality of the food, the ease with which digestion absorption and the rate at which assimilation of food takes place within the body system. It also enhances easy elimination of digestive waste from the body (Olumuyiwa et al., 2004). The proportion of moisture in this sample showed that it has high keeping quality and could be broken down easily within the body.

The moisture content (23.58±3.4) is lower than those reported for some common Nigeria leafy vegetables such as *Xanthosem saggilifolium* (14.7%), *Gnetum buchholsianium* (33.8%) but closer to that reported for *Vernonia*

amygdalina (27.4%) vegetable by Tunde (1998). The proportion of moisture in *Vernonia amygdalina* leaf protein concentrates showed that it has high keeping quality and could be broken down easily within the body.

Crude fat value is 9.20±0.85. This value is greater than 0.3% reported for *spinach* leaves, 0.4% reported for *chaya* leaves and 1.60% in *Amaranthus hybrids* leaves (Nwaogu et al., 2000). Fat in food is the principal source of energy but should not exceed the daily recommended dose of not more than 30 calories so as to avoid obesity and other related diseases (Gafar et al., 2011). One gram of lipid provides 8.37k cal (Asibey-Berko and Tayie, 1999), which indicates that 50g of *Vernonia Amygdalina* leaf protein concentrates would provide about 42k cal of energy.

The crude fibre content (10.46 ± 1.57) reported for *Vernonia amygdalina* leaf concentrate is high compared to 7.20% in sweet potato leaves, 1.3% *Tribulus terrestris* (*Tsaida*) leaves (Hassan and Umar, 2006). Dietary fibre helps to reduce serum cholesterol level, risk of coronary meat disease, colon and breast cancer and hypertension (Ganong, 2003).

The recommended daily allowance for fibre is 18.35g which indicate that 50g of *Vernonia amygdalina* leaf protein concentrates could not provide the daily fibre requirement of the body.

The proportion of crude protein content (44.28±2.43) is higher compared to the value reported for *Momordica foecide* leaves consumed in Swaziland and 11.29% on *basalm* apple (Hassan and Umar, 2006). This is expected for any plant materials that can find application in drug synthesis. Proteins are powerful compounds that build and repair body tissues and its essence is to furnish man with essential amino acids necessary for the maintenance of body tissue. The recommended dietary allowance (RDA) for children adult males and females are 28, 63 and 50 respectively. This indicates that *Vernonia amygdalina* vegetable leaf protein concentrate is a good source of daily proteins for children and adult females.

The ash concentration is 12.48 ± 0.24 which indicates that the leaves are rich in mineral elements. The value is higher compared to 1.8% reported for sweet potatoe leaves (Asibey-Berko and Tayie, 1999) and 5% in *Tribulus terrestris* leaves, but lower than 19.61% in *Amaranthus hybridus* leaves (Nwaogu et al., 2000).

Crude carbohydrate contents of the leaf protein concentrate of *Vernonia amygdalina* is 23.58±3.64 which indicates that this leaf protein concentrate is not a good source of energy since food carbohydrate is an important source of energy and dietary fibre. It also contributes to the sweetness, appearance and textural characteristics of many food substrates. The value is lower compared to some leafy vegetables like *Tribulus terrestris* 55.67% and 54.20% reported for water spinach leaves (Asibey-Berko and Tayie, 1999). The carbohydrate content can be boosted by mixing this leaf concentrate with foods that are richer in carbohydrate.

Table 2:Amino Acid Content of *Vernonia Amygdalina* Leaf Protein Concentrates

Amino Acid	Values
Lysine	5.23
Histidine	2.56
Arginine	5.51
Aspartic acid	8.65
Threonine	2.75
Serine	3.67
Glutamic acid	13.97
Proline	3.56
Glycine	1.83
Alanine	2.98
Cystine	1.76
Valine	5.21
Methionine	1.45
Isoleucine	4.74
Leucine	9.49
Tyrosine	4.56
Phenylalanine	3.38

Table 2 presents amino acid content of the leaf protein concentrate of *Vernonia amygdalina*. The sample contains significant quantity of glutamic acid (13.97), aspartic acid (8.65) and leucine (9.49).

The recommended daily allowance of glutamic acid is 21.6g for males and 20.0g for female. These values were higher than those obtained for *Vernonia amygdalina* vegetable leaf protein concentrates. This indicated that in order to meet up with the recommended daily allowance, significant quantity of these vegetable leaf protein concentrates would be consumed.

However, other values obtained for other amino acids are generally favourable compared with the FAO/WHO/UNU (1991) recommended daily allowance (Table 5).

From this table, the recommended value for lysine is 5.80 while 5.23 were observed for the leaf protein concentrates. The recommended value for methionine +

cystine is 2.50 while the observed value for *Vernonia amygdalina* is 3.21. Also, the recommended value for threonine is 3.40 which is higher than 2.75, recorded for the leaf concentrates. *Vernonia amygdalina* leaf concentrates appears to be better source of other amino acid in the reference table. Since the proportion in the table are lower than those observed for the leaf concentrates. The total essential amino acid value for *Vernonia amygdalina* is 37.37 while the non-essential amino acid is 41.93; the percentage proportion of total essential amino acid is 47.12% which is smaller compared to 52.88% of the non-essential amino acid.

Tryptophan is completely absent in *Vernonia amygdalina*, this is not a surprise because tryptophan is an essential amino acid which is obtainable from animal protein only and could be obtained by mixing with foods that are rich in tryptophan.

Table 3:Essential Amino Acid

	mg/100g
Leucine	9.49
Isoleucine	4.74
Lysine	5.23
Methionine	1.45
Phenyl alanine	3.38
Theonine	2.75
Tryptophan	ND
Valine	5.21
Histidine	2.56
Tyrosine	4.56
Selenocysteine	ND
Total	37.37

% Total Essential Amino Acid = 47.12%

NOTE N.D = NOT DETECTED.

Table 4: Non-Essential Amino Acid

Non-Essential Amino Acid	mg/100g
Alanine	2.98
Arginine	5.51
Aspartic acid	8.65
Cysteine	1.76
Glutamic acid	13.97
Glycine	1.83
Proline	3.56
Serine	3.67
Total	41.93

% Total Non-Essential Amino Acid = 52.88%

Table 5:FAO/WHO/UNU Reference Value of Amino Acid

Amino Acid	Reference Value
Lysine	5.8
Methionine + cystine	2.5
Threonine	3.4
Tryptophan	1.0
Valine	3.5
Leucine	6.6
Isoleucine	2.8
Phenyl Alanine + Tyrosine	6.3

Table 6:Concentration of Mineral Elements in *Vernonia amygdalina* Leaf Protein Concentrates

Mineral Elements	Concentration (mg/100g)
Na	57.5±0.34
Ca	151.6±1.40
K	61.5± 0.38
Mg	96.5±0.96
Fe	2.3±0.42
Zn	1.1±0.11
Mn	0.1±0.14
Se	ND
Cu	0.1±0.73
Pb	0.1±0.62

Key; ±; Mean triplicate determination, ND; Not detected

Table 6 presents the concentration of mineral elements in *Vernonia amygdalina* leaf protein concentrates.

Sodium content of *Vernonia amygdalina* leaf protein concentrates (57.5 ± 0.34 mg/100g) is high compared to 45 mg/100g reported for *Senna obtusifolia* and 5.00 ± 0.06 mg/100g reported for *Tribus terrestris* leaves (Hassan et al., 2005). *Vernonia amygdalina* can contribute 8.18% to Recommended Dietary Allowance. High sodium content of food is of great concern for health because too much of sodium could lead to high blood pressure. This result indicated that *Vernonia amygdalina* leaf protein concentrate could not lead to high blood pressure. That is it could be a good source of food for hypertensive patient.

Calcium content in *Vernonia amygdalina* leaf protein concentrates (151.6 ± 1.40 mg/100g) is high compare to 1.44 ± 0.06 , 1.13 ± 0.07 and 1.22 ± 0.05 reported for *Vernonia calvoana*, *Vernonia colorata* and

Vernonia calvoana vegetables species respectively by Ejoh et al. (2007) but lower than 9.41 mg/100g in *Mormordica balsamina* L leaves (Hassan and Umar, 2006). The Recommended Dietary Allowance (RDA) for adult men with 3000 kcal/day; recommended energy intake is 1,200 mg (NRC, 1989) and *Vernonia amygdalina* can only contribute 12.01% to the RDA. This indicates that *Vernonia amygdalina* vegetable leaf protein concentrates can contribute meaningful amount of dietary calcium which is required for growth, maintenance of bone, teeth and muscle by eating significant quantity.

Potassium value for *Vernonia amygdalina* is 61.5 ± 0.38 . The value is higher compared with 6.42 mg/100g found in *Diospyros mespiliformis* (L) and 14.55 ± 0.17 mg/100g reported for *L. astragalina* leave by Gafar et al. (2011) but lower compared to 220.00 ± 78 mg/100mg in *Cassia siamen* leaves (Ngaski, 2006). The sodium potassium ratio in the diet assists in the

prevention of hypertension and *arteriosclerotic* and for normal protein retention during growth stage.

The recommended daily allowance of potassium is 2000mg for adult (NRC, 1989) and *Vernonia amygdalina* can contribute 3.08% to recommended daily allowance meaning that the leaves can furnish body with very small quantity of dietary potassium.

Magnesium plays a vital role in calcium metabolism in bone. It is an important mineral element in connection with circulatory diseases such as ischemic heart disease. The magnesium content of the leaves protein concentrate is 96.5 ± 0.96 mg/100g. The value is high when compared with the 23.18 ± 0.4 mg/100g reported for *Amaranthus hybridus* leaves (Nwaogu et al., 2000) and 40.00 ± 0.00 mg/100g in *Cassia siamea* leaves (Ngaski, 2006). The recommended dietary allowance value for adult male is 350mg (NRC, 1989) and *Vernonia amygdalina* leaf protein concentrates contribute 27.57%.

The iron content of the *Vernonia amygdalina* is 2.3 ± 0.42 mg/100g. The value is closer to 2.80 ± 0.7 mg/100g reported for *T. terrestris* but higher than 1.9 ± 1.12 reported for *Celosia argentea* leaf protein concentrates (Sodamade et al., 2011) and those reported for some cultivated vegetables such as *spinach* (1.6mg/100g) *lettuce* (0.7 mg/100g) and *cabbage* (0.3 mg/100g) (Turan et al., 2003). Iron is required for haemoglobin formation and its deficiency leads to anemia. The recommended Dietary Allowance value for iron for male adult is 10-15mg (NRC, 1989), *Vernonia amygdalina* can contribute 23.0-15.33% of iron to RDA.

Zinc concentration of *Vernonia amygdalina* Leaf Protein Concentrates is 1.1 ± 0.11 , it was found to be higher compared to 0.02 mg/100g reported for *Diospyrus mespilliformis* (Hassan et al., 2004), and 0.10 ± 0.00 mg/100g reported for *Terrestris* leaves but lower when compared to 6.85 ± 1.00 mg/100g reported for *Talium triangulare* by Fasuyi (2006).

Zinc plays a vital role in gene expression regulation of cellular growth and participates as a co-factor of enzymes responsible for carbohydrates, proteins and nucleic acid metabolism (Gafar et al., 2011). The recommended Daily Allowance value of zinc for a male adult is 12-15 mg (NRC, 1989). *Vernonia amygdalina* leaf protein concentrates can provide 9.17-7.33% of zinc. The value indicated that this sample is a poor source of dietary zinc.

Manganese content in *Vernonia amygdalina* leaf protein concentrate is 0.1 ± 0.14 . The value is lower than 11.6 mg/100g reported for *Basalm* apple (Hassan and Umar, 2006). Manganese is one of the elements required in small concentration. It acts as in-activator of many enzymes.

Selenium was not detected in the sample while lead and copper were present in concentration of 0.1 ± 0.73 and 0.1 ± 0.62 respectively; copper plays a role in haemoglobin formation and it contributes to iron and energy metabolism. Too much of lead and *selenium* in food are not good in food since they can lead to food poisoning.

However, the proportions of these metals in the *Vernonia amygdalina* leaf protein concentrates are not in the concentration that could impair man health.

CONCLUSION

Leaf protein concentrates of *Vernonia amygdalina* reveals an interesting value of proximate composition and amino acid profile but leaf protein concentrates is not a food on its own but could be incorporated in food ingredients and various drug binders.

However, further research work could be investigated by using experimental animal to observe the dietary or nutritional implication of *Vernonia amygdalina* leaf protein concentrates; the functional properties and the mineral composition of this sample could also be evaluated.

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