Original Research Article

PROXIMATE, MINERAL, VITAMIN AND AMINO ACID ANALYSIS OF THE AERIAL PARTS OF *LEONURUS CARDIACA*

Abstract

This study evaluated the proximate, mineral, vitamin and amino acid composition of the aerial pats of *Leonurus cardiaca*. The moisture content was evaluated by drying at 105°C in an oven until a constant weight was reached, total ash, fibre, carbohydrate, and crude fat content were characterized through extraction with hexane, using a Soxhlet apparatus based on standard methods while crude protein was determined by the Kjeldahl method. Elemental analyses were carried out using an atomic absorption spectrophotometer, and flame photometer. Amino acid profile was evaluated using HPLC while precision and accuracy was evaluated using external and internal standards. The Mean and Standard deviation of the moisture content was 16.51±0.12%, crude protein (22.00±0.11%), crude fat (12.61±0. 12%), crude fibre (9.11±0. 06%), ash content (4.12±0. 06%), and carbohydrate (35.65±.06%). Thevitamin A concentration was 22.66±0.02mg/100g, vitamin E (16.07±0.01 mg/100g), Vitamin C (51.22±0.02mg/100 g), vitamin E (16.07±0.01 mg/100g), niacin (B3) (32.47±0.01mg/100g), thiamine (B1) (15.53±0.02mg/100g), and vitamin B_5 (31.91±0.02 mg/100g). The sodium concentration was 38.68±0.31 mg/100g, potassium (598.10±0.04 mg/100g), magnesium (496.74±0.19mg/100g), calcium (263.89±0.03mg/100g), copper (1.32±0.11mg/100g), iron (8.62±0.16mg/100g), zinc (4.41±0.27 mg/100g), manganese (2.12±0.41mg/100g), selenium (1.83±1.06mg/100g), and phosphorus (104.09±3.02mg/100g). The proximate, mineral, vitamin, and amino acid composition characterized showed *Leonurus cardiaca*possessa good nutritional values, hence justifies consumption of its aerial parts as vegetable in Southern and Eastern Nigeria.

Keywords: Amino acid, High performance liquid chromatography, *Leonurus cardiaca*, proximate, mineral, vitamin.

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1.0 INTRODUCTION

Several indigenous plants play a significant role in the diet of the populace [1] and are the cheapest, most available sources of important nutrients, supplying the body with minerals, vitamins, protein, energy and essential amino acids [2]. Medicinal plants are endowed with numerous substances that can be utilized for therapeutic purposes or for synthesis of drugs [3, 4].

WHF [5] stated that there over 789 million people in developing countries still suffers from malnutrition, especially infants and children of rural areas. Malnutrition can be tremendously reduced with an increased use of foods rich in energy, proteins, iron and vitamin A most especially those from the natural environment [2, 5]. The lack of nutritional information and inadequate development of nutritionally improved products from local raw materials have direct bearing on nutrition. Much effort has been concentrated on seeds while leafy vegetables have largely been ignored.

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Nigeria, increased interest has been observed in the use of natural plant products to improve health and it is considered as one of the first real functional food which has been enormously neglected food in the modern westernized diet [5,7]. Ignorance concerning the nutritional properties of food is the major reasons for under-utilization of natural plant products. There are a large number of Nigerians particularly from the Western, Southern, and Eastern parts of Nigeria who consume vegetables because of their flavors and taste, and have no consideration on their nutritional composition [8,7].

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Leonurus cardiaca (Motherwort) is probably best known as a uterine stimulant, which is where its name comes from. It is used for painful or delayed periods, and in the last few weeks of

pregnancy to prepare for childbirth. It is also known to ease symptoms of menopause [9]. It is used by trado-medical practioners for treatment of heart failure in traditional medicine. *Leonurus cardiaca* is a Latin name which means something like "lion's heart," and refers to motherwort's use as a cardiovascular tonic in traditional system of medicine. It strengthens the heart and can treat heart palpitations and irregularities, especially where those are associated with anxiety and tension. Preparation from *Leonurus cardiaca* is used as teas (Infusion). In the herbal medicine world, teas are called either infusions or decoctions. Infusions are made with non-boiling water, and are used for delicate plant material such as leaves or flowers. Decoctions are used for tough, woody material such as roots, bark, and twigs; and boil the plant material in order to extract the active constituents. Since the parts of motherwort that are used medicinally are leaves, flowers, and stems, it requires the infusion method [10].

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2.0 MATERIALS AND METHODS

2.1 Chemical/Reagents

All chemical/reagents used for this study were purchased from commercial industries and the manufacturers' standard methods and procedure were strictly followed with regard to this study

2.2 Source and Identification of Plant Material

The fresh aerial parts of *Leonurus cardiaca* (LNC) were harvested from Idema Community, in Ogbia Local Government Area of Bayelsa State, Nigeria. The plant sample was identified and authenticated by Dr. Ekeke Chimezie at the Herbarium Unit of the Department of Plant Science and Biotechnology (PSB), University of Port Harcourt. The sample was registered with Voucher Number UPH/P/203.

2.3 Proximate Analysis

The moisture content was evaluated by drying at 105°C in an oven until a constant weight was reached. For total ash determination, the aerial parts of the samples were weighed and converted to dry ash in a muffle furnace at 450 and at 550°C for incineration. The crude fat content was characterized through extraction with hexane, using a Soxhlet apparatus. All these determinations were carried out according to AOAC [11]. Kjeldahl method was used to quantify the crude protein concentration. Carbohydrate content was estimated by calculating the difference between the sums of all the proximate compositions from 100%. Energy values were obtained by multiplying the carbohydrate, protein and fat by the Atwater conversion factors of 17, 17 and 37, respectively [12].

2.4Minerals Analysis

Mineral analyses were carried out according to Martin-Prevel*et al*.[13]. Elemental analyses were carried out using an atomic absorption spectrophotometer and flame photometer to determine calcium, sodium, potassium and magnesium content. Iron, phosphorus, copper, lead, cobalt, nikel, molybdenum, iodine, selenium, manganese and zinc were determined calorimetrically. The concentration of each element in the leave sample was calculated on a dry matter basis.

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2.5Amino Acid Analysis

Amino acid analysis of plant sample was based on the Vázquez-Ortiz *et al*. [14] method. Powdered samples (5 mg) were hydrolyzed with HCl 6 M at 150°C during 6 h. After hydrolysis, the acid was removed by rotary evaporation (RE500 Yamato Scientific America Inc.). Sample was resuspended on 2 mL of sodium citrate buffer pH 2.2. Sample derivation was achieved adding o−phthalaldehyde (OPA) 7.5 mM to the sample on citrate buffer (OPA reagent contains β-mercaptoethanol and Brij). The HPLC method precision and accuracy was evaluated using

external and internal standards. The amino acid reference standard consisted on fifteen amino acids (0.05 µmoles mL-1 each amino acid) and was utilized to determine the retention times for each amino acid. As well, internal standard αaminobutyric (0.05 µmoles mL-1) was added to amino acid reference standard and each plant sample to normalize and quantify the amino acid content.

A gradient mobile phase of sodium acetate 0.1 M pH 7.2 and methanol (9:1) elute sample for amino acid separation trough C18 column reversed-phase octadecyl dymethylsilane particles (100 x 4.6 mm x 1/4" Microsorb 100-3 C18). Fluorescence detection was realized using an excitation-emission wavelength of 360 and 455 nm respectively. Star Chromatography work station (Varian version 5.51) software was used to achieve amino acid peak integration. The protein content of each plant was measured to determine amino acid recovery by HPLC using Micro Kjeldahl method [15].

2.6Vitamin Analysis

2.6.1 Preparation of Mixture Standard Vitamin Solutions

The stock standard solutions of vitamin C, B1, B3, B5 and B6 and were prepared by dissolving 25 mg of the each standard in 1 ml 0.1M hydrochloric acid in 25 ml standard volumetric flask. For preparation of standard stock solutions of vitamin B9 and B2, 25 mg of the each standard were dissolved in one ml 0.1 M sodium hydroxide in 25 ml standard volumetric flask. The standard solution was stored in amber-glass bottles in the refrigerator at 4 °C. The working standards were prepared by diluting with phosphate buffer (1M, pH 5.5).

2.6.2 Preparation of Sample Solution

Plant materials were washed with distilled water. The washed plant materials were cut into very small pieces, frozen in liquid nitrogen and kept at −20 °C until analysis. 1 g each of freeze-dried sample was soaked in 10 ml water and extracted with 1 ml 0.1M NaOH and 10 ml phosphate buffer (1M, pH 5.5) were added to it and kept in dark for 24 hours. The solution was first filtered through a Whatman No. 1 filter paper and the resulting filtrate was taken in a 25 ml volumetric flask and solution was topped up to the mark with HPLC grade water. The sample solution was filtered through 0.45 µm membrane filter before injection into LC system. The stock solutions of sample were kept in a refrigerator for further use.

2.6.3 Chromatographic Analysis of Water Soluble Vitamins

The chromatographic analysis was carried out following the method as described by Seal *et al.* [16] with minor modifications. The mobile phase contains acetonitrile (Solvent A) and aqueous trifluoro acetic acid (TFA, 0.01% v/v) (Solvent B), the column was thermostatically controlled at 220 C and the injection volume was kept at 20 µl. A gradient elution was performed by varying the proportion of solvent A to solvent B. Total analysis time per sample was 35 min. HPLC Chromatograms of all vitamins were detected using a photo diode array UV/detector at four different wavelength (290 nm) according to absorption maxima of analyzed compounds. Detection of compound was done in same manner that followed in detection of phenolic acids and flavonoids. The data were reported as means ± standard error of means of three independent analyses.

2.7 Statistical Analysis

All Data are represented as means ± standard deviation (M±S) were analyzed using Statistical Package foe Social Sciences (SPSS) for window version 17.0 USA. Descriptive statistics was done by one way analysis of variance (ANOVA) and multiple comparison was done using Turkey Post hoc at ($p \leq 0.05$) confidence interval.

3.0 RESULTS AND DISCUSSION

The proximate compositions determined in the aerial parts of the plan are summarized in Table 1. The Mean ± Standard deviation of the moisture content was27.63±0. 12 %, crude protein (22.00± 0.11 %), crude fat (12.61±0. 12 %), crude fibre (9.04±0. 06 %), ash content (4.12±0. 06 %), and carbohydrate (35.65±.06 %) as shown in Table 1. The moisture content (27.63±0. 12%) of the aerial parts shows that plant is a good source of water from vegetables for the cells of the body (Okeke *et al.*, 2008). The estimated carbohydrate content in the aerial parts of *Leonurus cardiaca* (35.65±.06 %) was high and carbohydrates are known to produce energy required for the body because they are essential nutrient required for adequate diet [17] and supplies energy to cells such as brain, muscle and blood [18]. The content of fat in the aerial parts of the plant (12.61±0. 12%) is within the range (8.3%-27.0%) reported for some leafy vegetables consumed in Nigeria [21]. Leafy vegetables are poor sources of lipids (19,20], therefore the increase in the consumption of vegetables would naturally lower fat intake. The protein content of the aerial parts of the plant was found to be very high (35.65±.06 %). Protein is vital for various body functions such as body development, maintenance of fluid balance, formation of hormones, enzymes and sustaining strong immune function [17], hence *Leonurus cardiaca*'s aerial parts is an excellent source of protein for body development, maintenance of fluid balance, formation of hormones, enzymes and sustaining strong immune function.

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Crude fiber content of this plant could aid in the absorption of trace elements in the gut and therefore increases intestinal bowel movement (Abolaji*et al.*, 2007). Consuming vegetables in diet could aid in managing constipation problems (Olowokudejo*et al.*, 2008).

Table 1 Proximate composition of the aerial parts of *Leonurus cardiaca*

Proximate	Concentration (%)
Moisture content	27.63±0. 12
Crude protein	4.12±0. 06
Crude fat	12.61±0. 12
Crude fibre	9.04±0. 06
Ash content	22.00±0.11
Carbohydrate	35.65±.06

Results are expressed as Mean ± Standard deviation. N=3

The result in Table 2 shows the presence of Vitamin A (22.66±0.02 mg/100g), Vitamin E (16.07±0.01 mg/100g), Vitamin C (51.22±0.02 mg/100 g), vitamin E (16.07±0.01 mg/100 g), niacin (B3) (32.47 0.01 mg/100 g), thiamine (B1) (15.53±0.02 mg/100 g), and vitamin B_5 (31.91±0.02 mg/100 g). Vitamin C and E are very important antioxidants which protect the cell membranes from oxidative stress/damage caused by free radicals [22]. The aerial parts of *Leonurus cardiaca* contain ascorbic acid and vitamin E, both of which are effective antioxidants. Vitamin C possesses an antioxidant property and required for maintenance of normal connective tissues, wound healing and also facilitates the absorption of dietary iron from the intestine [23]. The very high vitamin C content observed in the aerial parts of *Leonurus cardiaca* is indicative of the wound healing potential plant and its capacity to the absorption of dietary iron from the

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intestine. This also justifies the antioxidant activity of the leaves as reported by [23]. Deficiencies of these vitamins predispose the red cell membranes to damage leading to haemolysis [24]. Riboflavin and niacin are necessary for oxidative phosphorylation and for coenzyme formation respectively [24]. The aerial parts of *Leonurus cardiaca* contains moderate amount of vitamin A and therefore essential for clear vision.

Table 2 Vitamin Composition of the aerial parts of *Leonurus cardiaca*

Vitamins	Concentration (mg/100g)
Vitamin A	22.66±0.02
Vitamin E	16.07±0.01
Vitamin K	7.90±0.02
Vitamin B3	32.47 0.01
Vitamin B6	27.35±0.01
Vitamin B1	15.53±0.02
Vitamin B2	24.07±0.02
Vitamin B5	31.91±0.02
Vitamin C	51.22±0.02

Parameters were reported in mean and standard error (M±SE). n=3

Table 3 shows the mineral composition of the aerial parts of *Leonurus cardiaca*. The sodium concentration was 38.68±0.31 mg/100 g, potassium (598.10±0.04 mg/100 g), magnesium (496.74±0.19 mg/100 g)), calcium (263.89±0.03 mg/100 g), copper (1.32±0.11 mg/100 g), iron (8.62±0.16 mg/100 g), zinc (4.41±0.27 mg/100 g), manganese (2.12±0.41 mg/100 g), selenium (1.83±1.06 mg/100 g), phosphorus (104.09±3.02 mg/100 g), lead (0.21±3.00 mg/100 g), cadmium (0.03±0.04 mg/100 g)), cobalt (0.06±1.04 mg/100 g), nickel (0.06±0.17 mg/100 g), molybdenum (0.03±0.17 mg/100 g) and iodine (0.02±0.45 mg/100 g) as shown in Table 3. Potassium was observed to be higher in concentration followed by magnesium, calcium, phosphorus, sodium, iron, zinc, manganese, copper, selenium, lead, cadmium, cobalt, nickel, molybdenum while the least in concentration as iodine as shown in Table 3..

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The mineral compositions of the aerial parts of *Leonurus cardiaca* are shown in Table 3. It contains considerable amount of sodium (38.68±0.31 mg/100 g), potassium (598.10±0.04 mg/100 g), magnesium (496.74±0.19 mg/100 g), calcium (263.89±0.03 mg/100 g), copper (1.32±0.11 mg/100 g), iron (8.62±0.16 mg/100 g), zinc (4.41±0.27 mg/100 g), manganese (2.12±0.41 mg/100 g), selenium (1.83±1.06 mg/100 g), phosphorus (104.09±3.02 mg/100 g), lead (0.21±3.00 mg/100 g), cadmium (0.03±0.04 mg/100 g), cobalt (0.06±1.04 mg/100 g), nickel (0.06±0.17 mg/100 g), molybdenum (0.03±0.17 mg/100 g), and iodine (0.02±0.45 mg/100 g).

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Pathak and Kapil [25] reported that zinc is vital in protein synthesis, cellular differentiation and replication, immunity and sexual functions. Calcium is reported to be essential for blood clotting, bone and teeth formation and as a co-factor in some enzyme catalysis [26]. In humans, magnesium is required in the plasma and extracellular fluid, where it helps maintain osmotic equilibrium [27]. It can also prevent some heart disorders and lower blood pressure in humans. Iron facilitates the oxidation of biomolecules to control obesity, which predisposes an individual to various diseases. It is also essential for hemoglobin formation [27] and plays a role in energy transfer within the plant and also an essential constituent of certain enzymes and proteins. This justifies the use of *Leonurus cardiaca* in folklore medicine as a blood tonic because of its blood boosting effect [28].

The high quantities of sodium and potassium were present in the aerial parts of *Leonurus cardiaca* and these are principal cations of extracellular and intra-cellular fluids and aid in maintaining electrolyte balance in the body [26]. Potassium is essential and is required in large amounts for proper growth and plant reproduction. Phosphorous maintain blood sugar levels and

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normal heart contraction [29]. Potassium is also important for normal cell growth and repair, bone growth and kidney function. It plays an important role in maintaining the body's acid-alkaline balance [30]. Minerals found to be present in trace quantities in the aerial parts of *Leonurus cardiaca* are cadmium, nickel and lead. Cadmium and lead in high amounts are not ideal and not desirable for the functioning of the body.

Table 3 Mineral composition of the aerial parts of *Leonurus cardiaca*

Mineral elements	Concentration (mg/100)
Sodium	38.68±0.31
Potassium	598.10±0.04
Magnesium	496.74±0.19
Calcium	263.89±0.03
Copper	1.32±0.11
Iron	8.62±0.16
Zinc	4.41±0.27
Manganese	2.12±0.41
Selenium	1.83±1.06
Phosphorus	104.09±3.02
Lead	0.21±3.00
Cadmium	0.03±0.04
Cobalt	0.06±1.04
Nickel	0.06±0.17
Molybdenum	0.03±0.17
Iodine	0.02±0.45

Table 4 shows the mean and standard error (M±SE) of the amino acid composition of the aerial parts of *Leonurus cardiaca* in mg/100 g. The glycine concentration of the aerial parts of LNC was 4.94±0.03 mg/100 g while that of alanine was 4.98±1.17 mg/100 g, serine (2.92±1.23 mg/100 g), proline (4.83±0.31 mg/100 g), valine (4.22±0.11 mg/100 g), 4.30±0.15 mg/100 g), threonine (4.30±0.15 mg/100 g), isoleucine (4.82±0.01 mg/100 g), leucine (5.53±0.03 mg/100 g), aspartate (10.02±0.12 mg/100 g), lysine (5.60±0.07 mg/100 g), methionine (2.32±0.01

mg/100 g), glutamate (14.48±0.03 mg/100 g), phenylalanine (5.34±1.04 mg/100 g), histidine (5.37±1.00 mg/100 g), arginine (5.24±2.04 mg/100 g), tyrosine (4.51±0.00 mg/100 g), tryptophan (4.38±0.07 mg/100 g), and cystein (6.09±0.04 mg/100 g) as shown in Table 4. Glutamate has the highest concentration (14.48 g/100 g prot) followed by aspartate (10.02 g/100 g prot), lysine (5.60 g/100 g prot), histidine (5.37 g/100 g prot), phenylalanine (5.34 g/100 g prot), arginine (5.24 g/100 g prot), alanine (4.98 g/100 g prot), glycine (4.94 g/100 g prot), serine (4.92 g/100 g prot), isoleucine (4.82 g/100 g prot), proline (4.83 g/100 g prot), tyrosine (4.51 g/100 g prot), threonine (4.30 g/100 g prot), valine (4.22 g/100 g prot) and cystein ($6.08x10^{-1}$ g/100 g prot). The least in concentration was tryptophan ($4.38x10^{-1}$ g/100 g prot) while the sum total of the amino acid sub-members were 90.47 g/100 g prot) as presented in Table 4.

There few studies on quantitative amino acid analysis of medicinal plants. Reports in the literature are related to results of intake or topical functions, which derive from their use in traditional medicine [31]. In this study, eighteen amino acids in appreciable concentration were detected in the aerial parts of *Leonurus cardiaca* in aspartic acid, cystein, phenylalanine, leucine, valine, lysine, arginine, histidine, and glutamic concentrations were high. This is supported by the amino acid profile of *Rizophora mangle L* where protein/amino acid contents are dominated by acidic amino acids such as aspartic an glutamic acids, followed by neutral amino acids such as glycine [32].

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Aspartic acid function is essential for purine, pyrimidine, asparagine and inositol synthesis. Glutamic acid and glycine participate in the synthesis of glutathione increasing the antioxidant capacity of the plant. Valine maintains the balance of branched chain amino acids, whereas alanine is involved on hepatic autophagy, gluconeogenesis and transamination. Leucine regulates the protein turnover (mTOR signaling) and gene expression (33, 34]. The high concentration of

aspartic acid, glutamate, and glycine content in the aerial parts of Leonurus cardiaca could be responsible for the antioxidant capacity of the plant. Glycine, lysine, threonine and glutamate help to maintain intestinal integrity and health [35, 33, 36].Jutuki plant is used to treat stomach infections [37] sample, and it showed low amino acid concentration, some of which are related to bowel function, which somehow may explain the medicinal properties of the plant. This also justifies the use of formulations from Leonurus cardiaca for the treatment of stomach infection or gastrointestinal disorders.

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Table 4 shows the amino acid composition of the aerial parts of *Leonurus cardiaca.*

Amino acid	Concentration (g/100g prot)
Glycine	4.94±0.03
Alanine	5.98±1.17
Serine	2.92±1.23
Proline	4.83±0.31
Valine	5.22±0.11
Threonine	4.30±0.15
Isoleucine	4.82±0.01
Leucine	5.53±0.03
Aspartate	10.02±0.12
Lysine	5.60±0.07
Methionine	2.32±0.01
Glutamate	14.48±0.03
Phenylalanine	5.34±1.04
Histidine	5.37±1.00
Arginine	5.24±2.04
Tyrosine	4.51±0.00
Tryptophan	4.38±0.07
Cysteine	6.09±0.04

CONCLUSION

High concentrations of aspartic acid, glutamic acid, leucine, valine, lysine, phenylalanine, arginine, histidine, and cystein predominate in the aerial parts of *Leonurus cardiaca*. Carbohydrate and protein contents The high carbohydrate and protein contents observed in the aerial parts of *Leonurus cardiaca* are suggestive the plant could be an excellent source of energy and protein for body development, maintenance of fluid balance, formation of hormones, enzymes and sustaining strong immune function. The high vitamin C content observed in the aerial parts of *Leonurus cardiaca* is indicative of the antioxidant and wound healing potential plant and its capacity to the absorption of dietary iron from the intestine. The aerial parts of *Leonurus cardiaca* contains high amount of vitamin A and therefore essential for clear vision. The proximate, mineral, vitamin, and amino acid compositions characterized showed *Leonurus cardiaca*possess excellent nutritional values, hence justifiesconsumption its aerial parts as vegetable in Southern and Eastern Nigeria.

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NOTE

This study highlighted the effectiveness of “traditional medicine” which is an ancient tradition practiced in some parts of India. This ancient concept should be carefully investigated in the light of modern clinical science and can be adopted partially if considered appropriate

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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