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# Nutritional Characterization of *Combretum dolichopentalum* Leaves

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## Abstract

The nutritional value of *Combretum dolichopentalum* was determined by its proximate, mineral, amino acids and vitamin compositions. The macronutrients such as protein, lipid, moisture, fibre, ash were in varying concentration with carbohydrate recording the highest concentration (63.35 ± 4.93%). Elements present were potassium, sodium, phosphorus and iron amongst others. Vitamins A, E and B-vitamins, such as riboflavin, pyridoxine, folate, and biotin, were recorded, with niacin (2.26 ± 0.07 mg/ml) showing the highest concentrations. Leucine and glutamate were the highest essential and non essential amino acids respectively. Total aliphatic amino acid (TAAA) and Sulphur containing amino acid were recorded as 20.38 g/100 g and1.7 g/100 g respectively. The result showed that *C. dolichopentalum* possess nutritional potentials for energy metabolism and vital nutrients to maintain a state of desirable nutrition that could enhance the curative process of diseased conditions and maintenance of disrupted homeostatic status.

**Keywords:** Nutrients; Proximate; Amino acid; Vitamin profile; B-vitamins; Mineral elements; *Combretum dolichopentalum*

## Introduction

*Combretum dolichopentalum* make up the type genus of the family combretaceae. Several species are used in African and Indian traditional medicine. *C. dolichopentalum* commonly known as food for the small bird is one of the medicinal plants popularly used in treating disease conditions of the alimentary tract in Ibo ethnomedicine. *C. dolichopentalum* is used for the treatment of stomach ache, gastro intestinal disorders, such as dysentery, passage of bloody stool, diarrhoea and stomach ulcer in and around Ogwa in Imo State-Nigeria. Also, in Umuoshi Umunama in Ezinihitte Mbaise and other Mbaise ethnic nationality of Imo State, *C. dolichopentalum* is taken by women after parturition for reconditioning of the uterus after pregnancy. The leaves are cooked until the fluid content turns red, and is prepared as soup for drinking [1]. No one plant contains all nutrient needs. Thus a variety in diet is required among different foods. Whole grains, fruits and vegetables are among the foods most emphasized for our diet [2].

The body's nutritional health is determined by the sum of its nutritional status with respect to each needed nutrient. Three general categories are recognised; desirable nutrition, under nutrition, and over nutrition. Maintaining a state of desirable nutrition is the basis for establishing human nutrients and the diet plan to meet the required standard [3]. Once nutrient stores are depleted, a continuing nutritional deficit drains the body tissues. It is important to note that the body can only compensate to a certain point. This is because, when tissue concentrations of an essential nutrient fall sufficiently low, the body's metabolic processes eventually slow down or even stops. This response results from a biochemical lesion which develops in response to the nutrient deficiency. Diminished enzyme function often is the cause of the slowdown in biochemical function at the subclinical stage-where there are no signs or symptoms. For poor iron status, low concentration of haemoglobin are formed in the blood because the synthesis of haemoglobin requires iron.

If a biochemical deficit becomes severe, clinical signs and symptoms eventually develop and become outwardly apparent. It is then possible to note clinical lesions in the body perhaps in the skin, hairs, nails, tongue, or eyes. The complexion may become pale in case of iron deficiency and fatigue can quickly develop during even moderate activity. Both fat and water soluble vitamins are essential organic substances needed in small amounts in the diet for the normal function, growth, and maintenance of body tissues. The most prominent function is as co-factors in enzymatic reactions. The most distinguishing feature of vitamins in general is that they cannot be synthesized by human cells and, therefore, must be supplied in the diet. If the vitamin content is insufficient to meet needs, a deficiency occurs, accompanied by a measurable decline in health. Vitamins have proved useful as pharmacological agents in treating a limited number of nondeficiency diseases [3]. Nutritionally amino acids are of two types: essential amino acids; they are not synthesized by the organism and need to be supplied in the diet (e.g methionine, threonine, tryptophan, valine, isoleucine, leucine, phenylalanine, and lysine) and non essential amino acids; these can be synthesized by the body and may not be requisite component of the diet. There is a third group of semi- essential amino acids; these are growth promoting factors, since they are not synthesized in sufficient quantity during growth. They include arginine and histidine. They become essential in growing children, pregnancy, and lactating women. Amino acids serve variety of functions such as been glucogenic, specific amino acids give rise to specialized products such as thyroid hormones, catecholamines, melanin, niacin, serotonin (tryptophan), creatine, bile salts, glutathione, neurotransmitters, haem, pyrimidines and purines. In this note, it becomes rational to exploit the nutritional properties of plants especially those that are easily accessed at all seasons such as *C. doilichopentalum* to remedy issues food security such as malnutrition especially in areas where poverty is the reason for low standard of living.

## Materials and Methods

### Plant sample

Fresh leaves *of C. dolichopentalum* were harvested from Obinze in

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Owerri West Local Government Area of Imo State, Nigeria. The plant was authenticated by Mr Ozioko, of the Bioresource Development and Conservation Program (BDCP), Research Centre University of Nigeria, Nsukka in Enugu State.

## Biochemical analysis

**Proximate composition**: Total lipid, protein, ash, and moisture contents were carried out as described by Association of Official Analytical Chemicals (AOAC) [4]. The total lipid content consist of both free and bound lipid constituent, extraction was done by the use of Soxhlet exhaustive extraction method.

Determination of the protein, briefly; Two grams (2.0 g) of the sample were measured into a kjeldahl flask containing boiling chips, copper and sodium sulphate catalyst. Concentrated $H_2SO_4$ and 5 glass beads were into the flask. The digest was cooled and its content poured into 50.0 ml of cold distilled water in an Erlenmeyer flask placed in an ice bath. The content of the flask was transferred to a measuring cylinder and made up to 200.0 ml. A blank was prepared in the same manner without the sample. 50.0 ml of the diluted digest was placed in a clean dry Kjeldahl flask. Furthermore, 50.0 ml of 0.1 N $H_2SO_4$ acid solutions was poured into a beaker with 3 drops of methyl orange indicator. The flask was connected to complete a distillation set up and 50.0 ml of 40% NaOH solution was released into the Kjeldahl flask. The distillation process was allowed to run until the total volume in the receiver beaker was 90.0 ml. A burette was filled with 0.1 M $H_2SO_4$. The same procedure was carried out for the blank.

Moisture content was determined from the measurement of the mass lost due to evaporation of water at or near the boiling point of water.

**Mineral composition**: Calcium, Potassium and Sodium concentrations were determined on a Jenway Digital Flame Photometer (PFP7 Model). Phosphorus was determined routinely by the vanado-molybdate spectrophotometric method. Mineral such as magnesium, iron and zinc were analyzed using a Varian AA20 Atomic Absorption Spectrophotometer according to the method of American Public Health Association [5] after ashing the pulverized leave sample.

**Determination of Calcium, Potassium, Sodium and Phosphorus briefly:** The ash of the samples was digested with 5 ml of 2 M HCl in a crucible and heated to dryness on a heating mantle. Another 5 ml of 2 M HCl was added, heated to boil and filtered into a 100 ml volumetric flask. The filtrate was made up to mark with distilled water. The concentration of calcium, potassium and sodium was determined with the Jenway Digital Flame Photometer (PFP7 Model) using the filter corresponding to each mineral element. To determine phosphorus, 10 ml of the filtrate was added into 50 ml flask and 10 ml of Vanadate yellow solution was added. The flask was made to mark with distilled water, stoppered and left for 10 minutes for full yellow colour development. The concentration of phosphorus was obtained by measuring absorbance of the solution on a Spectronic 20 spectrophotometer at a wavelength of 470 nm.

Determination of Mg, Fe, Zn. Briefly; into 2 g of the plant sample was added 200 ml of the acid mixture (650 ml concentrated $HN0_3$; 80 ml perchloric acid; 20 ml concentrated $H_2S0_4$). This was heated until a clear digest was obtained. The digest was diluted with distilled water to 100 ml and appropriate dilutions were made for each element. Concentration of elements were conducted using Varian AA20 Atomic Absorption Spectrophotomeer

**Vitamin Content**: Quantitative determination of vitamin A, B1 (Thiamine), B2 (Riboflavin), B3 (Nicotinic acid), B6 (Pyridoxine) B7 (Biotin), B9 (Folate) and vitamin E (Tocopherol) contents of *C. dolichopentalum* were determined according to the method of Eitenmiller and Landen, [6] and AOAC. [4].

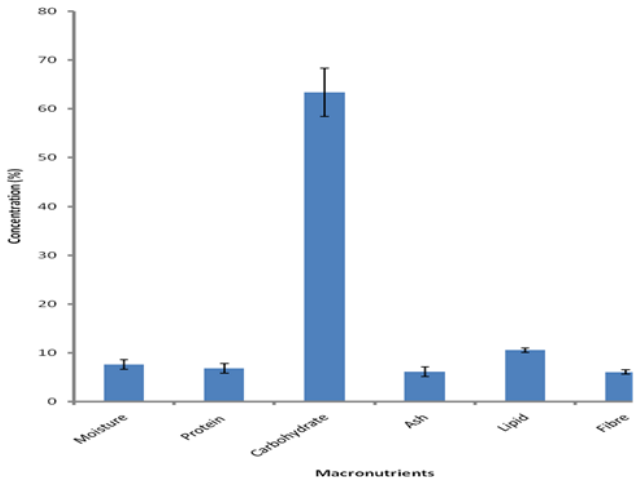
**Amino acid composition**: determined by the method described by Benitez [7]. Briefly; the pulverized plant sample (200 mg) was wrapped in filter paper and defatted in a Kjeldhal flask. Concentrated $H_2S0_4$ (10 ml) and a catalyst mixture (0.5 g) containing sodium sulphate ($Na_2SO_4$), copper sulphate ($CuSO_4$) and selenium oxide ($SeO_2$) in the ratio of 10:5:1 was added into the flask to facilitate digestion. The defatted sample was hydrolysed with 7 ml of 6 M HCl in a glass ampoule. The glass ampoule was sealed with Bunsen burner flame and put in an oven preset at 105°C ± 5°C for 22 hours. Ten microlitre of the hydrolysate was loaded into the cartridge of the Technicon sequential Multi-Sample Amino Acid (TSM) analyzer.

## Statistical Analyses

Some of the data obtained in this study were expressed as mean ± standard deviation of triplicate determinations, and others in percentages.

## Results and Discussion

Malnutrition is widespread in Nigeria affecting all age groups in varying degrees with clinical symptoms of tissue wasting, growth failure, body weakness and weakened immune competence. The use of lesser known plants for food by peasants in the face of economic downturn affecting developing countries needs to be encouraged [8]. The nutritional composition studies on *C. dolichopentalum* leaves showed presence of carbohydrates, protein, lipids, crude fibres, ash and moisture at appreciable percentages (Figure 1). The highest and lowest concentrations were recorded in carbohydrate (63.35 ± 4.93%) and ash (6.18 ± 0.98%) respectively (Figure 1). *C. dolichopentalum* contains fairly good quantities of carbohydrates, proteins and lipids than *Boerhavia diffusa* (10.56%, 2.26% and 1.16% respectively) and *Commelina nudiflora* (5.67%, 1.69% and 1.44% respectively) [9], as well as *Phyllanthus amarus* (45.52%, 6.10% and 6.03% respectively) [10]. The good distribution of nutrients in *C. dolichopentalum* should encourage domestic animals to graze on them [9]. However, this is not so with *C. dolichopentalum* leaves, because domestic animals have been observed




Bars represent mean± standard deviation of triple determinations

**Figure 1:** Proximate composition of *C. dolichopentalum* leaves.

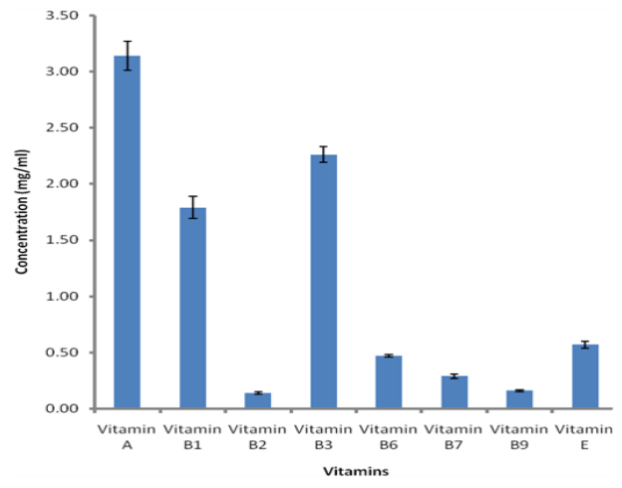
to avoid it. This could be attributed to the plants phytochemical constituent such as the alkaloid and the saponin contents. Saponin has been observed to protect plants from protozoa and molluscs and other predator species [11]. The plant leaves have a higher ash content (6.18 ± 0.98%) compared to *Spondias mombin* leaves (0.09 ± 0.01%) [12] and *Persea amaericana* (2.50 ± 0.01%) [13], but less moisture content (Table 1) when compared to *S. mombin* leaves and *Persea amaericana* seed. Ash content of material is the residue remaining after ignition at 500 – 600°C for 2-4 hours. Thus it is a reflection of the mineral content of a plant.

The values obtained in the mineral studies (Table 1) shows high concentration of sodium (193.8 ± 10.05 mg/kg) and potassium (223.24 ± 13.66 mg/kg). Appreciable amount of iron (0.88 ± 0.41 mg/kg) and phosphorus (0.52 ± 0.07 mg/kg) besides others were recorded. Minerals are known to play important metabolic roles in the living cells [14]. Sodium and potassium are the major macroelements found in *C. dolichopentalum* leaves (Table 1). Reports showed that they were also higher than those found in *S. mombin* leaves [12]. The combined presence of sodium and potassium are involved in acid-base balance, pyruvate kinase and $Na^+/K^+$-ATPase activities. Thus *C. dolichopentalum* extract could enhance carbohydrate (energy) metabolism and maintenance of cell's membrane potential. The use of extracts of *C. dolichopentalum* leaves can contribute potassium within the established adequate intake (AI) level, which has been found to lower blood pressure, reduce salt sensitivity and minimize the risk of kidney stones. Therefore extracts *of C. dolichopentalum* leaves may add as a veritable source of potassium which has reduced in modern day diet, thereby reducing the risk of stroke and some chronic diseases [15,16].

| Minerals | Concentration (mg/kg) |
|---|---|
| Sodium | 193.8 ± 10.05 |
| Potassium | 223.24 ± 13.66 |
| Magnesium | 0.06 ± 0.01 |
| Calcium | 0.05 ± 0.01 |
| Phosphorus | 0.52 ± 0.07 |
| Zinc | 0.12 ± 0.02 |
| Iron | 0.88 ± 0.41 |

Values are mean ± standard deviation of triple determinations

**Table 1:** Mineral composition of *C. dolichopentalum* leaves.




Bars represent mean± standard deviation of triple determinations

**Figure 2:** Vitamin composition of *C. dolichopentalum* leaves.

However, magnesium, calcium, phosphorus, zinc and iron contents of the leaves of *C. dolichopentalum* were low when compared to earlier cited common edible vegetables. Iron, zinc and manganese strengthen the immune system possibly as either antioxidants or apoenzymes of enzymatic antioxidants. The iron and glycine content could be attributed for its use after parturition since these are necessary for the haemoglobin formation. Other minerals identified include phosphate and chloride. Phosphorus plays a role in the formation of the structure of the bone and tooth; it is a component of membrane phospholipids, ATP, DNA and RNA.

Vitamin composition of *C. dolichopentalum* (Figure 2) shows ample presence of vitamin A (3.14 ± 0.13 mg/ml), vitamin B1 (1.79 ± 0.10 mg/ml), vitamin B2 (0.14 ± 0.01 mg/ml), vitamin B3 (2.26 ± 0.07 mg/ml), vitamin B6 (0.47 ± 0.01 mg/ml), vitamin B7 (0.16 ± 0.01 mg/ml), vitamin B9 (0.16 ± 0.01 mg/ml), and vitamin E (0.57 ± 0.3 mg/ml). Vitamins are coenzymes in several energy yielding chemical reactions. The appreciable concentration of B-vitamins implies that *C. dolichopentalum* may be important in energy metabolism. Vitamin B1 (Thiamine) acts as coenzyme to pyruvate dehydrogenase, α–ketoglutarate dehydrogenase and transketolase in translocation reaction in hexose monophosphate pathway (HMP). Deficiency of vitamin B1 results in Beriberi [3].

Deficiency of vitamin B2 causes glossitis, heilosis, seborrheic dermatitis etc [17]. Heliosis is the cracking of the tissues around the mouth, this shows that the plant plays a role in healing of tissues, and this may be one of the reasons women after parturitions use it to heal tissues damaged during delivery. The presence of vitamin B3 (Niacin) shows that the plant extract could facilitate energy production, prevent pellagra, characterised by dermatitis, diarrhoea and dementia [18]. Vitamin B6 presence implies that consumption of *C. dolichopentalum* extract could prevent anaemia and used for the treatment of epilepsy and hypertension via production of Gamma-amino butyric acid (GABA) [19].

Vitamin B7 (Biotin) shows that pregnant women and nursing mothers may consume the plant extract to enhance growth foetus and the infant respectively and as well prevent convulsions, a challenging illness of the infant. The vitamin B9 (Folate) content is important for pregnant women especially in the first trimester, to prevent anaemia, neural tube defects in foetus [20,21]. This reiterates the beneficial potentials of *C. dolichopentalum* in pregnancy. The vitamin A of *C. dolichopentalum* may be important in preventing night blindness, diminished neutrophils and follicular hyperkeratosis [22]. Also, the vitamin E (Tocopherol) content indicates ability to ameliorate lipid peroxidation and enhance activities of reproductive cells in male organisms [23,24]. Furthermore, the amino acid profile (Table 2) shows appreciable concentration of essential and non essential amino acids; leucine and glutamate were the most abundant essential and non-essential amino acids respectively.

Total Sulphur containing amino acid (TSAA) content was (1.7 g/100 g). Both Cysteine and methionine (Table 3) are sources of sulphur. Cysteine forms disulphide bonds which play a special role in the structure of many proteins by forming covalent links between parts of a protein molecule or between different polypeptide chains [19]. Methionine in S–adenosylmethionine (active methionine) by transmethylation transfers methyl group to various substances. Total amino acid content was 57.8 g/100 g (Table 3). This is higher than that reported by Ibegbulem et al., [25] for *Eliaeis guineensis* and *Raphia hooker*i, but the total neutral amino acids (TNAA) (Table 3) were less. The 'R' chains of neutral amino acids such as alanine, valine, leucine and

isoleucine tend to cluster together within proteins; stabilizing protein structure by means of hydrophobic interactions. The hydroxyl group of tyrosine can form hydrogen bonds- an important functional group in some enzymes. Tyrosine forms thyroid hormones, epinephrine, norepinephrine and melanin.

Glutamate, cysteine and glycine, synthesizes glutathione. This signifies that the plant extract could also enhance the free radical scavenging activity of glutathione peroxidase (GPx) *in vivo*. Acidic amino acids such as glutamate and Aspartate (15.65 g/100 g) (Table 3) are used for the synthesis of pyrimidines, purines and Gamma amino butyric acid (GABA) [19].

| Amino Acids | Concentration (g/100 g protein) |
|---|---|
| **Essential amino acids** | |
| Lysine (Lys) | 2.89 ± 0.02 |
| Histidine (His) | 2.02 ± 0.07 |
| Arginine (Arg) | 4.06 ± 0.03 |
| Valine (Val) | 3.91 ± 0.08 |
| Methionine (Met) | 0.91 ± 0.03 |
| Isoleucine (Ileu) | 3.11 ± 0.08 |
| Leucine (Leu) | 5.84 ± 0.14 |
| Phenylalanine (Phe) | 3.63 ± 0.08 |
| Tryptophan (Trp) | ND |
| **Non essential amino acids** | |
| Aspartic Acid (Asp) | 7.32 ± 0.03 |
| Threonine (Thr) | 2.24 ± 0.05 |
| Serine (Ser) | 3.01 ± 0.04 |
| Glutamic acid (Glu) | 8.33 ± 0.04 |
| Proline (Pro) | NC |
| Glycine (Gly) | 3.84 ± 0.11 |
| Glutamine (Gln) | NC |
| Alanine (Ala) | 3.68 ± 0.016 |
| Cystine (Cys) | 0.79 ± 0.06 |
| Tyrosin (Try) | 2.22 ± 0.08 |

ND = Not detected, NC = Not computed. Values are mean ± standard deviation of triplicate determination

**Table 2:** Amino acid profile of *C. dolichopentalum* leaves.

| Parameter | Concentration |
|---|---|
| total amino acids (TAA) | 57.80 |
| Total non essential amino acid (TNEAA) | 33.25 |
| Total essential amino acid with His | 24.55 |
| Total essential amino acid without His | 22.53 |
| Total neutral amino acids (TNAA) | 25.63 |
| Total acidic amino acids (TAAA) | 15.65 |
| Total basic amino acid (TBAA) | 6.95 |
| Total sulphur-containing amino acids (TSAA) | 1.70 |
| Total aromatic amino acids (TArAA) | 5.85 |

**Table 3:** Total amino acids and amino acid groups (g/100 g protein) in leaves of *C. dolichopentalum.*

| Parameter | Concentration |
|---|---|
| % Total non essential amino acids (%TNEAA) | 57.8 |
| % Total essential amino acids (%TEAA)with His | 42.47 |
| % Total essential amino acids (%TEAA) without His | 38.98 |
| % Total neutral amino acid (%TNAA) | 44.34 |
| % Total acidic amino acids (%TAAA) | 27.08 |
| % Total basic amino acids (%TBAA) | 12.02 |
| % Total sulphur-containing amino acid (%TSAA) | 2.94 |
| % cys in TSAA | 46.47 |
| % Total aromatic amino acid (%TArAA) | 10.12 |
| % tyr in TArAA | 37.95 |

**Table 4:** Percentages of amino acid groups and amino acids in leaves of *C. dolichopentalum*

| Parameter | Concentration |
|---|---|
| Leu/Ile ratio | 1.88 |
| TBAA/TAAA ratio | 0.44 |
| TEAA/TAA ratio with His | 0.42 |
| TEAA/TAA ratio without His | 0.39 |

**Table 5:** Ratio of amino acids and amino acid grouping in leaves of *C. dolichopentalum*

*C. dolichopentalum* leaves is observed to contain basic amino acids (6.95 g/100 g) (Table 3). The most hydrophilic 'R' groups are those that are either positively (basic) or negatively (acidic) charged. Total essential amino acids with histidine and without histidine are 24.55 g/100 g and 22.53 g/100 g respectively. In many enzyme catalysed reaction, histidines residue facilitates the reaction by serving as a proton donor/acceptor. Histidines also in this capacity act as a buffer in plasma [19] Although *C. dolichopentalum* contains more TNEAA than TEAA (with or without His) (Table 3), their essential amino acid (EAA) contents are higher than the 1.7 g EAA content of the egg white as reported by Connolly. This indicates that the amino acid (AA) content would support protein synthesis. TArAA which includes Phe and Tyr (Table 3) was higher than that reported by Nelson et al., [24] and Phe value was higher than Tyr value (Table 2). Phe enhances protein synthesis and it is a standard antisickling amino acid, which exhibits synergistic activity in any extract with other antisickling components, drugs and nutrients when compounded together, and has found pronounced role in the management of sickle cell disease [26-28].

The % TNAA of the plant was below 50% (Table 4) implying an overall charged proteins. The % TAAA and % TBAA (Table 4) indicates a more negatively charged amino acids unlike *Elaeis guineensis* and *Raphia hookeri* wines [24]. The less than one (<1) value calculated for TBAA/TAAA ratio (Table 5) confirms the acidic amino acids content of *C. dolichopentalum* and thus could be acidic at physiological pH. This does not differ from *P. Africana* seeds [29,30]. TSAA showed the lowest value in amino acid groupings (Table 3). % cystine content (Table 4) was higher than the 7.68 ± 0.37 and 13.14 ± 0.83 reported for *Elaeis guineensis* and *Raphia hookeri* wines [24]. Synthesis of glutathione may be limited by the availability of cystine [31]. It therefore follows that consumption of *C. dolichopentalum* extracts, could help keep the thiol redox status in the reduced state.

The Leu/Ile ratio (Table 5) showed more Leu than Ile content. Although diets with high leucine content impairs tryptophan and niacin metabolism [30], our result showed high niacin content. However, Trp was not reported, because usually they were destroyed during acid hydrolysis of amino acid determination [32,33].

## Conclusion

These findings provide quantitative estimation of the proximate, vitamin, amino acids, as well as mineral element analyses which are important in understanding the nutritional benefits of unpopular plants. The appreciable concentration of minerals, vitamins, carbohydrates and amino acids in the leaves *C. dolichopentalum* is nutritionally important. It implies that the plants hold tremendous promise in providing variable macro-micronutrients that could enhance the curative process of ill health and maintenance of homeostasis. Further work on extraction and purification of active constituents should be of interest.

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