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# Amino acid quality indices of the leaves of *Clerodendrum volubile*

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ABSTRACT

**Objective:** To evaluate the amino acid profile and quality indices of *Clerodendrum volubile* (*C. volubile*) leaves.
**Methods:** Dried leaves of *C. volubile* were blended, defatted and subjected to amino acid analysis using the technicon sequential multi-sample amino acid analyzer. The amino acid quality indices which covers for chemical score, essential amino acid index, nutritional index, true digestibility, protein digestibility corrected amino acid score, and digestible indispensable amino acid score were evaluated using standard formulas.
**Results:** Amino acid analysis revealed glutamic acid to have the highest concentration, with cysteine having the least. Aspartic acid had the highest chemical score, this was followed by glycine, histidine and arginine, respectively. The least scores were observed in serine and methionine. Glutamic acid had the highest value for true digestibility and protein digestibility corrected amino acid score, with the least observed in cysteine. Digestible indispensable amino acid score evaluation showed histidine to have the highest value for infants (birth to 6 months), threonine for children (6 months to 3 years), while isoleucine was observed to have the highest value for older children, adolescents and adults. The essential amino acid index value was less than 4, while nutritional index value was less than 0.5.
**Conclusions:** These results indicated the leaves of *C. volubile* as a potential source of amino acids in the human diet as portrayed by its amino acids profile and qualities.

## 1. Introduction

Over the years malnutrition has been recognized and continues to remain a major public health challenge in the third world, with Sub-Saharan Africa being the most hit[1,2]. It is been estimated that malnutrition alone accounts for over half of childhood mortality annually[3]. Diets deficient in macronutrients and micronutrients have been identified as the chief culprit, particularly protein and amino acids. Proteins and amino acids have been shown to be fundamental in nutritional intervention. This can be attributed to their ability to preserve active tissue (protein) mass as well as other variety of functions[4]. Malnutrition can thus be tremendously reduced by improved consumption of diets rich in energy, proteins, iron and vitamin A most especially those from the rural environment. However, a dearth of nutritional information and inadequate development of nutritionally improved products from local raw materials have been shown to have direct bearing on nutrition[5]. Much focus has been on nuts while leafy vegetables, to a large extent, have been ignored.

Leafy vegetables have been recognized as general good sources of nutrients and constitute a major component of most West African diets[6]. These vegetables constitute diverse varieties, with most being underutilized. Of such vegetables is *Clerodendrum volubile* (*C. volubile*) commonly consumed in South-West Nigeria. It is locally called “obenetete” by the Urhobos and Itsekiri of the Niger Delta. It is referred to as magic leaf owing to its use in the treatment and management of several ailments which include diabetes, ulcer, arthritis, rheumatism, dropsy, amongst others[7]. Erukainure *et al*.[5] reported the phytochemical properties of the leaves as well as its *in vitro* antioxidant activities. They further reported the nutritional qualities of the leaves indicating high contents of vitamin A, manganese, iodine, and zinc[5]. They also reported a moderate

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biological values of the leaves, but higher values of true digestion, protein efficiency ratio and net protein utilization[8].

To the best of our knowledge, this is the first time that the amino acid qualities of the leaves of *C. volubile* are being reported. Therefore, this study aims at reporting the amino acid profile of *C. volubile* leaves as well as investigating its quality indices.

## 2. Materials and methods

### 2.1. Plant materials

Fresh leaves of *C. volubile* were purchased from local farmers in Ifon, Nigeria. They were air-dried and grounded to fine powder. The blended samples were stored in air-tight containers at room temperatures for laboratory analysis.

### 2.2. Nitrogen determination

The nitrogen content of the sample was determined using the Association of Official Analytical Chemists methods[9].

### 2.3. Defatting of sample

A total of 4 g of the sample was subjected to soxhlet extraction using chloroform/methanol mixture (2:1) for 15 h[9].

### 2.4. Determination of amino acid profile

The amino acid profile of the sample was determined according to the method described by Benitez[10]. The defatted sample was hydrolyzed, evaporated in a rotary evaporator and loaded into technicon sequential multi-sample amino acid analyzer.

### 2.5. Amino acid quality indices

Chemical score was calculated using the Food and Agriculture Organization (FAO)/World Health Organization (WHO) formula (FAO/WHO 1990). Essential amino acid index was calculated by the method described by Oser[11]. Nutritional index was calculated using the formula presented by Crisan and Sands[12]. True digestibility was calculated according to the method described by Addis *et al.*[13]. Protein digestibility corrected amino acid score (PDCAAS) was calculated using the method described by Sarwar and McDonough[14] using the essential amino acid composition of the test sample and the amino acid pattern suggested by FAO/WHO[15] with slight modifications. Digestible indispensable amino acid score (DIAAS) was calculated using the FAO prescribed method[16].

### 2.6. Statistical analysis

To address the biological variability, each set of experiments was repeated at least three times. Statistical significance was established using One-way ANOVA, and data were reported as mean ± SD. Significant difference was established at $P < 0.05$. Statistical analyses were carried out using SPSS for Windows, version 15.0 (SPSS Inc., Chicago, USA).

## 3. Results

The amino acid profile and chemical scores of *C. volubile* leaves are shown in Table 1. The amino acids concentrations were observed to be less than 11 g/100 g protein, with glutamic acid having the highest concentration. This was followed by aspartic acid and leucine, respectively. Methionine and cystine were observed to have the least concentration. Aspartic acid had the highest chemical score, this was followed by glycine, histidine and arginine, respectively. The least score were observed in serine and methionine.

**Table 1**
Amino acid contents and chemical score of the leaves of *C. volubile*.

| Amino acids | Concentration (g/100 g protein) | Chemical score |
|---|---|---|
| Lysine | 4.21 ± 0.09 | 90.73 ± 5.20 |
| Histidine | 2.19 ± 0.01 | 129.59 ± 4.50 |
| Arginine | 5.62 ± 0.04 | 123.79 ± 10.45 |
| Aspartic acid | 8.68 ± 0.10 | 142.53 ± 8.00 |
| Threonine | 3.61 ± 0.02 | 105.87 ± 5.90 |
| Serine | 3.41 ± 0.03 | 56.18 ± 2.89 |
| Glutamic acid | 10.09 ± 0.08 | 92.65 ± 9.80 |
| Proline | 3.36 ± 0.01 | 115.07 ± 15.20 |
| Glycine | 4.02 ± 0.04 | 139.10 ± 10.25 |
| Alanine | 3.81 ± 0.07 | 69.40 ± 5.00 |
| Cystine | 1.11 ± 0.03 | 59.04 ± 8.50 |
| Valine | 4.05 ± 0.07 | 67.28 ± 3.78 |
| Methionine | 1.18 ± 0.02 | 39.20 ± 11.20 |
| Isoleucine | 3.00 ± 0.09 | 60.00 ± 10.11 |
| Leucine | 7.54 ± 0.80 | 110.88 ± 11.40 |
| Tyrosine | 2.98 ± 0.02 | 92.83 ± 7.50 |
| Phenylalanine | 4.22 ± 0.05 | 85.43 ± 6.89 |

Values were expressed as mean ± SD; $n = 3$.

The percentage comparison of essential amino acid from *C. volubile* leaves to the reference protein (egg) showed higher values for leucine and threonine, with methionine having the least value (Figure 1).

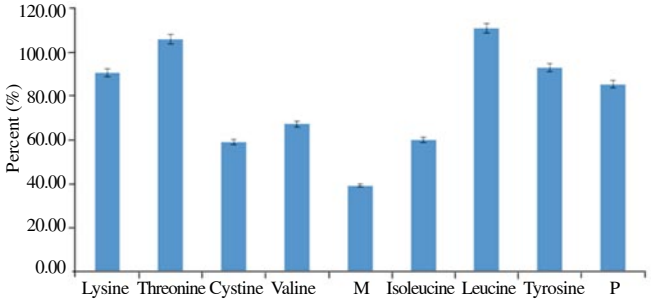


**Figure 1.** Percentage comparison of the essential amino acid composition of *C. volubile* leaves with the reference protein (egg) (sample/standard multiply by 100)%.
Values were expressed as mean ± SD; $n = 3$; M: Methionine; P: Phenyalanine.

Glutamic acid was observed to have the highest value for true digestibility and PDCAAS as depicted in Table 2. This was followed by aspartic acid, leucine and arginine, respectively, with the least values observed in methionine and cysteine. DIAAS of *C. volubile* leaves revealed histidine to have the highest value for infants (birth to 6 months) as depicted in Figure 2. This was followed by threonine, while isoleucine had the lowest. For children (6 months to 3 years), threonine had the highest value, this was followed by leucine, with lysine having the least. Isoleucine was observed to have the highest value for older children, adolescents and adults. This was followed

by histidine and threonine respectively, while lysine had the lowest.

**Table 2**
True digestibility and PDCAAS of the leaves of *C. volubile.*

| Amino Acids | True digestibility | PDCAAS |
|---|---|---|
| Lysine | 3.76 ± 0.25 | 34.15 ± 0.50 |
| Histidine | 1.96 ± 0.09 | 25.37 ± 1.00 |
| Arginine | 5.02 ± 0.01 | 62.20 ± 0.87 |
| Aspartic acid | 7.76 ± 0.02 | 11.06 ± 0.10 |
| Threonine | 3.23 ± 0.03 | 34.17 ± 0.08 |
| Serine | 3.49 ± 1.80 | 17.13 ± 0.11 |
| Glutamic acid | 9.20 ± 0.22 | 83.58 ± 3.62 |
| Proline | 3.00 ± 0.01 | 34.57 ± 0.99 |
| Glycine | 3.59 ± 0.03 | 49.99 ± 1.01 |
| Alanine | 3.41 ± 0.05 | 23.64 ± 0.34 |
| Cystine | 0.99 ± 0.01 | 5.86 ± 0.09 |
| Valine | 3.62 ± 0.18 | 24.36 ± 0.01 |
| Methionine | 1.06 ± 0.09 | 4.14 ± 0.04 |
| Isoleucine | 2.68 ± 0.03 | 16.09 ± 0.50 |
| Leucine | 6.74 ± 0.22 | 74.74 ± 0.80 |
| Tyrosine | 2.66 ± 0.01 | 24.73 ± 0.90 |
| Phenylalanine | 3.77 ± 0.06 | 32.23 ± 0.87 |

Values were expressed as mean ± SD; $n = 3$.

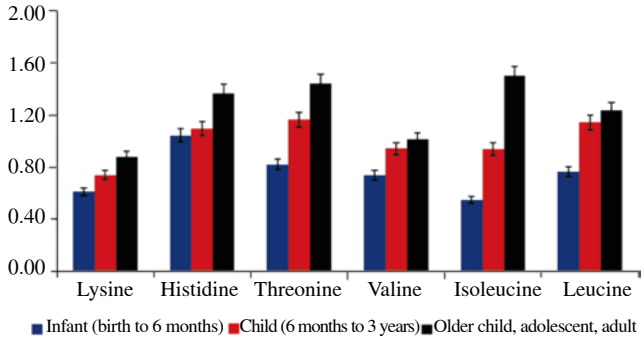


**Figure 2.** DIAAS of the leaves of *C. volubile*.
Values were expressed as mean ± SD; $n = 3$.

The essential amino acid index of the leaves was observed to be (3.300 ± 0.020) as shown in Table 3. The nutritional index was observed to be (0.370 ± 0.001).

**Table 3**
Amino acid quality indices of the leaves of *C. volubile.*

| Quality index | Values |
|---|---|
| Essential amino acid index | 3.300 ± 0.020 |
| Nutritional index | 0.370 ± 0.001 |

Values were expressed as mean ± SD; $n = 3$.

## 4. Discussion

The role of amino acids in the nutrition and well-being of an individual has long been recognized. They are not only recognized to be the building block of protein but also play major physiological role such as regulation of key metabolic pathways gene expression, cell signaling and key precursors for syntheses of hormones[17]. Therefore, optimal balance of amino acid in the diet will not only aid growth and eliminate malnutrition but aid in whole body homostasis. In this study, the amino acid profile and quality indices of *C. volubile* leaves were investigated with the aim of improving its utilization in malnutrition management as well as total well-being improvement.

The high content of glutamic acid in the leaves (Table 1) is of tremendous benefits as its functions have been documented in several studies. They are major substrate for protein synthesis, anabolic precursor for muscle development and maintain the acid-base balance in the kidney[18]. The essential amino acids present in the leaves is of tremendous health benefits as it compared very well with the reference protein (Figure 1). Essential amino acids have long been recognized as an important component of the human diet owing to their inability to be synthesized *de novo* by the body. Isoleucine, leucine and valine are regarded as branched chain amino acids and play major physiological roles aside nutrition[17,19]. Their supplementation has been shown to promote protein synthesis as well as its turnover[19]. The antioxidant properties of methionine against free radicals have been reported[20]. Erukainure *et al.*[21] and Adefegha and Oboh[22] reported the free radical scavenging activities of the leaf extract. The observed methionine content of the leaf was rather low but may however, contribute to the reported antioxidant activities. The observed amino acid profile of the leaves is therefore of significant nutrition and health benefits as it would not only boost growth and development but improved health.

The amino acid score is an important tool for predicting protein quality in terms of the potential capacity of the food protein to provide the correct pattern of dietary essential amino acids[16]. As shown in Table 1, the limiting amino acid score was observed to be methionine. Omoyeni *et al.*[23] reported similar observations for *Amaranthus cruentus, Corchorus olitorius, Cnidoscolus chayamansa, Thuja occidentalis* and five other indigenous leafy vegetables commonly consumed in South-West Nigeria. Being a vegetable, these leaves are often major ingredients in traditional dishes and thus the observed limiting amino acid (methionine) can be provided by the other ingredients present in the diets.

Digestion and absorption are considered inherent parts of protein quality and used to predict protein quality[24]. In this study, the observed values for the amino acids were less than 10 (Table 2). Erukainure *et al.*[8] however, reported a rather higher value for crude protein of *C. volubile* leaves in Albino male rats. These low values may be attributed to the type of fibres and polysaccharides present in the leafy vegetables[24] as high fibre contents of leafy vegetables have been reported in several studies. Anti-nutritional factors have also been shown to reduce protein and amino acids digestion and bioavailability[25].

There have been increasing interests in the metabolic effects of specific individual dietary amino acids, taking into consideration the amounts of digestible or preferably bioavailable amino acids in foods and proteins[16]. Over the years PDCAAS has been adopted for the assessment of dietary protein quality for human, with emphasis on the individual amino acids[26]. In this study, it was observed that the PDCAAS values were dependent on the individual amino acid concentration and as such a function of the true digestibility values (Table 2).

However, the shortcomings of PDCAAS have been identified and have been a major debate in determining the determining protein quality in human diets. Its major shortcomings include overestimation of protein quality of products containing anti-nutritional factors, non-consideration of the bioavailability of amino acids, and its overestimation of protein qualities that are limiting in more than one amino acid[16]. The DIAAS has been recommended by FAO[16] to replace PDCAAS. In this study, the DIAAS for individual

amino acids of *C. volubile* leaves increased with age groups (Figure 2). A DIAAS over 100 for an individual food designates a potential by the food to supplement that of another food with a lower DIAAS value[16]. Thus, the high percentage of histidine observed for all age groups indicates the leaf as a potential supplement to other foods with lower DIAAS. Same for threonine and leucine for both children (6 to 3 years) and older children.

The essential amino acid index and nutritional index have been recognized as useful tools to rapidly evaluate food formulations for protein quality[27]. The observed value for *C. volubile* leaves (Table 3) was rather higher that reported for raw, blanched and fermented *Moringa oleifera* lam leaves[28]. Thus, indicating the leaf as a good source of protein.

Results from this study indicated the leaves of *C. volubile* as a potential source of amino acids in the human diet as portrayed by its amino acids profile and qualities.

## Conflict of interest statement

We declare that we have no conflict of interest.

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