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Comparative Amino Acid Compositions of *Curculigo pilosa* Root and *Citrullus colocynthis* Fruit Bark

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Abstract

The study evaluated comparative amino acids compositions of *Curculigo pilosa* roots and *Citrullus colocynthis* fruit bark. The amino acids compositions were determined using amino acid analyzer. The results of amino acid compositions showed that eighteen amino acids were detected in both samples. Ten of the detected amino acids were essential amino acids and eight were non-essential. Glumatic acid had the highest concentration of 11.20 and 11.98 g/100g protein in *Curculigo pilosa* roots and *Citrullus colocynthis* fruits respectively. Leucine was the second highest concentrated amino acid in *Curculigo pilosa* roots and *Citrullus colocynthis* fruits with the values of 8.17 and 7.24 g/100g protein respectively. Histidine was the third highest amino acid in *Curculigo pilosa* roots while *Citrullus colocynthis* fruits had very low histidine level. The fourth highest amino was arginine in both samples. The percentage coefficient of variance (CV %) of the amino acid values were generally low with the exception of histidine with CV % value of 88.98 while rest of CV % values ranged from 0-26.92 showing the closeness of the amino acid values in the two samples to each other. Total amino acids (TAA) for *Curculigo pilosa*

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roots and *Citrullus colocynthis* fruits were 78.92 and 72.47 g/100g protein while the total essential amino acids (TEAAs) of *Curculigo pilosa* roots and *Citrullus colocynthis* fruits are 41.21 and 34.04 g/100g protein respectively. Percentage cysteine in (total sulphur amino acids) TSAA were 22.52 and 30.32 g/100g protein for *Curculigo pilosa* roots and *Citrullus colocynthis* fruits respectively. The results of this study indicate that *Curculigo pilosa* roots and *Citrullus colocynthis* fruits are rich in essential amino acid while their % Cysteine/TSAA values were relatively low.

Introduction

Medicinal plants constitute an effective source of both traditional and modern medicine. These plants have been shown to have genuine utility and about 80% of the rural population depends on them as primary health care (Akinyemi [1]). Plants have been used as sources of remedies for the treatment of many diseases since ancient times and people of all continents especially Africa have this old tradition. Despite the remarkable progress in synthetic organic medicinal products of the twentieth century, over 25% of prescribed medicines in industrialized countries are derived directly or indirectly from plants (Newman et al. [2]). However, plants used in traditional medicine are still understudied (Kirby [3]). In developing countries, notably in West Africa, new drugs are not often affordable. Thus, up to 80% of the population uses medicinal plants as remedies (Kirby [3]).

Citrullus colocynthis and *Curculigo pilosa* belong to a different family but are use as traditional medicine to most diseases (Kim et al. [4]). This is perennial herbs usually trailing. Commonly found wild in the sandy lands of North West (Denton and Adeiran, [5]). Also found indigenous in Arabia, West Asia, and Tropical Africa and in the Mediterranean region. It originally bore the scientific name *Colocynthis citrullus*, but is now classified as *Citrullus colocynthis* (Denton and Adeiran [5]). They are generally called Egusi in Yoruba land and *Citrullus colocynthis* is specifically called Egusibara. The genus *Curculigo pilosa* belong to the family *hypoxidaceae* and consist of approximately 20 species of exclusively tropical origin (Kocyan [6]). The members of the family are small to medium herbs, with grass-like leaves and an invisible stem, modified into corm or rhizome. The rhizomes of *Curculigo pilosa* was the first African species to be described of *curculigo* genus (Palazzino et al. [7]). The rhizomes of this plant possess medicinal properties and are used as food. It is traditionally used in the manufacture of infant food and sorghum beer in West Africa. The presence of high

amylolytic activity in extracts of curculigo pilosa explains its traditional use in the preparation of easily digestible infant food and in the traditional method for the preparation of sorghum beer (Dicko et al. [8]).

The amino acids found in nature occur either in free form or as linear chains in peptides and proteins. Analysis of amino acids plays a significant role in the study of the composition of proteins, foods, foodstuffs and other materials of biological origin. In multi-cellular organisms, most of the proteins are based on L-amino acids that have a great influence in both human and animal nutrition, health maintenance, and possess potent therapeutic applications (Ambrogelly et al. [26]). Plants subjected to different environmental and physiological stresses can accumulate amino acids in their system that play pivotal role in combating the stress. The amino acids produced in plant systems act as osmolyte, regulate ion transport, modulate stomata opening, activate phytohormones and growth substances, generate chelating effect on micronutrients and play a vital role in the detoxification of heavy metals. They are also responsible for the synthesis and functional properties of specific enzymes, gene expression, and redox-homeostasis (Zhao et al. [27]). Most importantly, in higher plants the amino acids serve as precursors for secondary metabolism (Zhao et al. [27]). Thus, the amino acids are directly related to plant stress physiology and have diverse preventive and recovery effects.

Information on the nutrient composition of locally available plants is very scanty and where available, these data are related to only the most popular plants. These plants use for herbal purpose call for more investigative data on the nature of their composition and properties. Some of this plants back and root have been neglected and not given a pride of place in the herbal medicine of the world people, which might be a result of ignorance of their nutritive value and other possible benefit of these plants.

Aim and objectives

The study was designed to evaluate and compare the amino acid compositions of *Citrullus colocynthis* fruit bark and *Curculigo pilosa* root.

Materials and Methods

Materials

The equipment, chemicals and reagents used in this study were of analytical grade and quality.

Collection and identification of plant materials

Fresh fruits of *Citrullus colocynthis* were collected from Umuka-Okposi, Ohaozara Local Government Area, Ebonyi State; while *Curculigo pilosa* roots were collected from Shaki town of Oyo state; and were identified by a taxonomist, Professor S. C. Onyekwelu in the Department of Biological Sciences, Ebonyi State University, Abakaliki, Ebonyi State.

Methods

Preparation of plant materials

The fresh fruit bark of *Colocynthis citrullus* and *Curculigo pilosa* were cut into pieces and air-dried in the laboratory at room temperature for two weeks. The dried samples were pounded to certain texture using pestle and mortar. The samples were further milled into fine powder using a mechanical grinder, sieved and stored in an air tight plastic container for further analysis.

Determination of amino acid compositions of Citrullus colocynthis fruit bark and Curculigo pilosa root

The amino acid profile was determined using methods described by Benitez [28].

Defatting sample

The sample was defatted using method described by (AOAC [29]).

Nitrogen determination

A small amount (0.115 mg) of ground sample each was weighed and wrapped in whatman filter paper (No.1) and put in the Kjeldhal digestion flask. Concentrated sulphuric acid (10 ml) was added. 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. Four pieces of anti-bumping granules were added. The flask was then put in Kjeldhal digestion apparatus for 3 hours until the liquid turned light green. The digested sample was cooled and diluted with distilled water to 100 ml in standard volumetric flask. Aliquot (10 m1) of the diluted solution with 10 ml of 45% sodium hydroxide was inserted into the Markham distillation apparatus and distilled into 10 ml of 2% boric acid containing 4 drops of bromocresol green/methyl red indicator until about 70 ml of distillate was collected. The distillate was then titrated

with standardize 0.01 N hydrochloric acid to grey coloured end point.

$$\text{Percentage Nitrogen} = \frac{(a-b)\times 0.01\times 14\times V}{W\times C}\times 100$$

where:

a = Titre value of the digested sample

b = Titre value of blank sample

V = Volume after dilution (100ml)

W = Weight of dried sample (mg)

C = Aliquot of the sample used (10ml)

14 = Nitrogen constant in mg.

Hydrolysis of the sample

Thirty grams of each defatted sample was weighed into glass ampoule. Seven ml of 6 N of Hydrochloric acid (HCl) was added and oxygen was expelled by passing nitrogen into the ampoule (this is to avoid possible oxidation of some amino acids during hydrolysis, e.g., methionine and cystine). The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at $105^0C \pm 5^0C$ for 22 hours. The ampoule was allowed to cool before broken open at the tip and the content was filtered to remove the humins. It should be noted that tryptophan is destroyed by 6 N hydrochloric acid during hydrolysis. The filtrate was then evaporated to dryness using rotary evaporator. The residue was dissolved with 5 ml of acetate buffer (pH 2.0) and stored in plastic specimen bottles, which were kept in the freezer.

Loading of the hydrolysate into analyzer

The amount loaded was 60 micro litres. This was dispensed into the cartridge of the analyzer. The analyzer is designed to separate and analyze free acidic, neutral and basic amino acids of the hydrolysate. Method of Calculating Amino Acid Values: An integrator attached to the Analyzer calculates the peak area proportional to the concentration of each of the amino acids.

Determination of tryptophan

The tryptophan in the known sample was hydrolyzed with 4.2 N sodium hydroxide

(Joseph [30]). Sample defatting and nitrogen determination were done as described above.

Hydrolysis of the sample

Thirty grams of the defatted sample was weighed into glass ampoule. Ten ml of 4.2M NaOH was added and oxygen was expelled by passing nitrogen into the ampoule. The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at 105°C ± 5°C for four hours. The ampoule was allowed to cool before broken open at the tip and the content was filtered to remove the humins. The filtrate was neutralized to pH 7.00 and evaporated to dryness at 40°C under vacuum in a rotary evaporator. The residue was dissolved with 5ml of borate buffer (pH 9.0) and store in plastic specimen bottles, which were kept in the freezer.

Loading of the hydrolysate into TSM analyzer

The amount loaded was 5 micro litres. This was dispended into the cartridge of the analyzer. The period of an analysis lasted for 76 minutes.

Method of calculating amino acid values

An integrator attached to the analyzer calculates the peak area proportional to the concentration of each of the amino acids. Method of calculating amino acid values from the chromatogram peaks: The net height of each peak produced by the chart recorder of TSM (each representing an amino acid) was measured. The half-height of the peak on the half-height was accurately measured and recorded. Approximate area of each peak was then obtained by multiplying the height with the width of half-height. The known sample was dried, grinded to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Applied Biosystems PTH Amino Acid Analyzer.

Calculation of other protein quality parameters

Determination of the ratio of total essential amino acids (TEAA) to the total amino acids (TAA), i.e. (TEAA/TAA), total sulphur amino acids (TSAA), percentage cystine in TSAA (% Cys/TSAA), total aromatic amino acids (TArAA), total neutral amino acids (TNAA), total acidic amino acids (TAAA) and total basic amino acids (TBAA) were estimated from the results obtained for amino acids profiles.

Results

The results showed that eighteen amino acids were detected in the both samples as shown in Table 1. Glumatic acid had the highest concentration of 11.20 and 11.98 g/100g protein in *Curculigo pilosa* roots and *Citrullus colocynthis* fruits respectively as shown in Table 1. Leucine was the second highest concentrated amino acid in *Curculigo pilosa* roots and *Citrullus colocynthis* fruits with the values of 8.17 and 7.24 g/100 g protein respectively while aspartic acid was the third highest, histidine were the fourth highest amino acid in *Curculigo pilosa* roots and *Citrullus colocynthis* fruits had very low histidine level. The fifth highest amino was arginine. The CV % of the amino acid values were generally low with the exception of histidine with CV % value of 88.98 while rest of CV % values ranged from 0-26.92 showing the closeness of the amino acid values in the two samples to each other. Total amino acids (TAA) for *Curculigo pilosa* roots and *Citrullus colocynthis* fruits were 78.92 and 72.47 g/100g protein as shown in Table 2 while the TEAAs of *Curculigo pilosa* roots and *Citrullus colocynthis* fruits 41.21 and 34.04 g/100g protein. Percentage cysteines in TSAA were 22.52 and 30.32 g/100g protein for *Curculigo pilosa* roots and *Citrullus colocynthis* fruits as shown in Table 3. Tables 2-3 shows the concentrations of total acidic AA (TAAA), total neutral AA (TNAA), total sulphur AA (TSAA), total aromatic AA (TArAA) and their percentage levels.

Table 1. Amino acid composition of *Curculigo pilosa* root and *Citrullus colocynthis* fruit bark dry weight in g/100 g protein.

Amino Acids	***Curculigo pilosa* root**	***Citrullus colocynthis* fruit bark**	**Mean**	**S.D**	**CV%**
*Leucine	8.17	7.24	7.71	0.66	8.53
*Lysine	3.08	3.16	3.12	0.06	1.92
*Isoleucine	3.47	3.34	3.41	0.10	2.93
*Phenylalanine	4.97	4.08	4.53	0.63	13.91
*Trytophan	0.92	0.63	0.78	0.21	26.92
*Valine	3.95	4.09	4.02	0.10	2.49
*Methionine	1.10	1.23	1.17	0.09	7.69

Proline	3.25	2.94	3.10	0.23	7.42
*Arginine	6.19	5.59	5.89	0.42	6.79
Tyrosine	3.27	3.27	3.27	0.00	0.00
*Histidine	6.20	1.41	3.81	3.39	88.98
Cystine	0.91	1.21	1.06	0.21	19.81
Alanine	3.94	4.48	4.21	0.38	9.03
Glutamic acid	11.20	11.98	11.58	0.54	4.66
Glycine	3.52	3.61	3.57	0.06	1.68
*Threonine	3.16	3.27	3.22	0.08	2.48
Serine	3.51	3.70	3.61	0.13	3.60
Aspartic acid	8.13	7.26	7.70	0.62	8.05

*= Essential Amino acid

Table 2. Concentrations of essential, non-essential, acidic, neutral, and sulphur, aromatic Amino ACID of *Curculigo pilosa* root and *Citrullus colocynthis* fruit bark dry weight in g/100g protein.

Amino Acids	***Curculigo pilosa* root**	***Citrullus colocynthis* fruit bark**	**Mean**	**S.D**	**CV%**
TAA	78.92	72.47	75.71	4.56	5.94
TEAA	41.21	34.04	37.63	5.10	13.55
TNEAA	37.71	38.43	38.07	0.51	1.34
TNAA	24.15	23.99	24.07	0.11	0.45
TAAA	19.33	19.22	19.23	0.08	0.42
TBAA	15.47	10.16	12.82	3.76	29.33
TSAA	2.01	2.44	2.23	0.30	13.45
TArAA	9.16	7.98	8.57	0.83	9.69
TAA/TEAA	1.92	2.13	2.03	0.15	7.38

Total amino acid (TAA), Total essential amino acid (TEAA), Total non-essential amino acid (TNAAA), Total sulphur amino acids (TSAA), Total aromatic amino acids (TArAA), Total neutral amino acids (TNAA), Total acidic amino acids (TAAA) and Total basic amino acids (TBAA)

Table 3. Percentage concentrations of essential, non-essential, acidic, neutral, and sulphur, aromatic amino acid of *Curculigo pilosa* root and *Citrullus colocynthis* fruit bark dry weight in g/100g protein.

Amino Acids	***Curculigo pilosa* root**	***Citrullus colocynthis* fruit bark**	**Mean**	**S.D**	**CV %**
%TEAA	52.22	46.97	49.60	3.71	7.48
%TNEAA	47.78	53.03	50.41	3.71	7.36
%TNAA	30.60	33.10	31.85	1.77	5.56
%TAAA	24.49	26.52	25.51	1.44	5.65
%TBAA	19.60	14.02	16.81	3.95	23.93
%TSAA	2.55	3.67	3.11	0.79	25.40
%TArAA	11.61	11.01	11.31	0.42	3.71
% Cysteine in TSAA	22.52	30.32	26.42	5.52	20.89

% = percentage

Total amino acid (TAA), Total essential amino acid (TEAA), Total non-essential amino acid (TNAAA), Total sulphur amino acids (TSAA), Total aromatic amino acids (TArAA), Total neutral amino acids (TNAA), Total acidic amino acids (TAAA) and Total basic amino acids (TBAA)

Discussion and Conclusion

Discussion

From the Table 1, glumatic acid had the highest concentration of 11.20 and 11.98 g/100g protein in *Curculigo pilosa* roots and *Citrullus colocynthis* fruits respectively followed by leucine with the values of 8.17 and 7.24 g/100 g protein in *Curculigo pilosa* roots and *Citrullus colocynthis* fruits respectively. Other amino acids recorded in decreasing order are aspartic acid, histidine and arginine. Apart from histidine with CV % value of 88.98, CV % of the amino acid values were generally observed to be low. Total amino acids (TAA) for *Curculigo pilosa* roots and *Citrullus colocynthis* fruits were 78.92 and 72.47 g/100g protein while the TEAAs of *Curculigo pilosa* roots and *Citrullus colocynthis* fruits were 41.21 and 34.04 g/100g protein respectively. Percentage cysteines in TSAA recorded 22.52 and 30.32 g/100g protein for *Curculigo pilosa* roots and *Citrullus colocynthis* fruits respectively.

This is in line with the report of Olorunfemi et al. [9] which reported that *Moringa oleifera* leaves had the highest level of total amino acids (76.4 g/100g) and followed by the root (70.9 g/100g) while stem had 65.4 g/100g. The result also supported the report of Omoyeni et al. [32] which revealed a high value of glutamic acid, aspartic acid and leucine and low level of cystine, histidine, methionine and serine in dried leaves of *Melanthera scandens*. Another study on Amino acid compositions of *Luffa cylindrica* seed (Oyetayo and Ojo [10]) showed that *Luffa cylindrica* seed contains a high proportion of essential amino, glutamic acid was the most abundant non-essential acid found while arginine was the most concentrated essential amino acid. Aremu et al. [11] made a similar observation in *L. cylindrica* seed kernel. Osibona et al. [12] reported that the most abundant amino acids in *Clarias gariepinus and Tilapia zillii* the two fish species were glutamic acid, aspartic acid, leucine and lysine ranging from 9.49% to 18.16%. Oyetayo and Ojo [10] reported that the total amino acid concentration was 72.71g/100g protein and the total essential amino acid concentration was 38.76g/100g protein in *Luffa cylindrical* seed flour.

Igwenyi et al. [13] reported that the concentration of arginine in *Irvigna gabonesis* and *Citrullus colocynthis* were 5.01 and 9.32 g/100g protein respectively. The concentration of histidine was 2.40 and 4.77 g/100g for *Irvigna gabonesis* and *Citrullus colocynthis* respectively (Igwenyi et al. [13]). The seeds were also high in non-essential amino acids such as cysteine in *Irvigna gabonesis* while glutamic acid and aspartic acid were high in both seeds. The common culture of combining soups made with these seeds will effectively compensate for deficiencies in the balance of nutrients especially, the limiting essential amino acids in both seeds. The result of this study was not in correlation with the report of Aja et al. [14] which reported the presence of nineteen (19) amino acids with proline (0.89 %) and tryptophan (0.78 %) as the major amino acids in ethanol-fruit extract of *Phoenix dactylifera fruit* (Dates fruit) sold in Abakaliki, Ebonyi State, Nigeria. Cysteine was not detected (Aja et al. [14]).

Arginine (1.77-8.22 g/100g crude protein) is essential for children and reasonable levels were present in the two samples: the lysine contents of the samples (2.17-3.53 g/100g cp) were about one half to the content of the reference egg protein (6.3 g/100 g), and any of the samples will therefore serve as an average source for the amino acid. The study revealed that TEAA in *Uvaria chamae* stem bark was 34.41 g/100g protein which is comparable to some literature values of nonconventional meat sources (g/100g protein): 35.1 for *Zonocerus variagatus* (Adeyeye [15]); 35.0 for *Macrotermes*

bellicossus (Adeyeye [15]); 42.8 for *Limicolaria sp*., 36.1 for *Archatina archatina*, 45.0 for *Archachatina marginata* (Adeyeye and Afolabi [16]); 38.6 for heart and 42.2 for liver of African giant pouch rat (*Cricetomys gambianus)* (Adeyeye and Aremu [17]) while that of Ruzu bitters was 17.44 g/100 g protein. The contents of TSAA were generally lower than the 5.8 g/100 g cp recommended for infants (FAO/WHO [18]). The TArAA range suggested for ideal protein (6.8-11.8 g/100 g) FAO/WHO [18] has present values for *Curculigo pilosa* roots and *Citrullus colocynthis* fruits 9.16 and 7.98 g/100 g cp. The TArAA are precursors of epinephrine and thyroxin (Robinson [19]).

The percentage ratios of TEAA to the TAA in the samples were 52.22 % for *Curculigo pilosa* roots and 46.97 % for *Citrullus colocynthis* fruits which were strongly comparable to that of egg (50 %) (FAO/WHO [24]), 43.6 % reported for pigeon pea flour (Oshodi et al. [20]), 43.8-44.4 % (beach pea protein isolate) (Chavan et al. [31]), 46.2 % (liver) and 46.3 % (heart) reported for African giant pouch rat (*Cricetomys gambianus*) (Adeyeye and Aremu [17]). The percentage ratios of TEAA to the TAA in the samples were well above the 39 % considered to be adequate for ideal protein food for infants, 26 % for children and 11 % for adults (FAO/WHO [18]). Most animal proteins are low in cystine (Cys) and hence in Cys in TSAA. For examples, (Cys/TSAA) % were 36.3 in *M. bellicosus* (Adeyeye [15]); 25.6 in *Z. Variegates* (Adeyeye [15]); 35.5 in *A. marginata*, 38.8 in *A. archatina* and 21.0 in *Limicolaria sp*., respectively (Adeyeye and Afolabi [16]); 23.8- 30.1 % in three fresh fish consumed in Nigeria (Adeyeye [15]) and 29.8 % in *Gymnarchus niloticus* (Trunk fish).

In contrast, many vegetable proteins contain substantially more Cysteine than Methionine, for examples, 62.9 % in coconut endosperm (Adeyeye [15] and Aremu et al. [11]); its range is 58.9-72.0 in guinea corn (Adeyeye et al. [21]); it is 50.5 % in cashew nut 43; it is 40.7 % in *Triticum durum* (Adeyeye [22]) and 44.4 % in *Parkia biglobosa* seeds (Adeyeye [23]). In the present study % (Cysteine/TSAA) values ranged from 22.53-30.32 in both samples which were much lower to the usual plant values. Thus, for animal protein, Cysteine is unlikely to contribute up to 50 % of the TSAA (FAO/WHO [24]). The % Cysteine /TSAA had been set at 50 % in rat, chick and pig diets (FAO/WHO [24]) but not in man. Cysteine can spare with Met in improving the protein quality and has positive effects on mineral absorption, particularly zinc (Mendoza [25]).

Conclusion

The study showed that *Curculigo pilosa* roots and *Citrullus colocynthis* fruits had

reasonable levels of total amino acid and essential amino acids and low % Cys/TSAA values.

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