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Investigations of Amino Acids Profile of Three Selected Indigenous Vegetable Leaf in Ohaukwu Local Government Area Ebonyi State Nigeria

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Abstract: Amino acids play important roles in human health , variation in composition of amino acids and other metabolites in the body leads to deterioration in health status. However , our knowledge of amino acid from plants are still limited. This study was undertaken to evaluate the amino acid content in selected vegetable plants in Ohaukwu local government area of Ebonyi State using standard analytical techniques. The results of amino acids profile in *Pterocapus santalinoide, Ficus sur* and *Vitex doniana* showed total amino acid composition (74.26%, 66.86 % and 189.26 %), total essential amino acid (36.97%, 33.5, 76.71 %), total non essential amino acid (37.29, 33.36, 112.55%), total neutral amino acid (11.82, 9.89, 29.26 %), total acidic amino acid (19.65, 17.09, 21.6%), total basic amino acid (11.82 %, 9.89%, 29.26 %), total sulfur amino acid (2.32, 1.75, 1.76 %) respectively. Results of our study suggest the great value of these plant species for use in pharmacy, food industry and phytotherapy. It provide valuable data for the establishment of nutritional databases. Hence, amino acids were present in *Pterocapus santalinoide, Ficus sur* and *Vitex doniana*. The total content of amino acid was found to be of good measure, which further guides in drug development from these plants. Based on this information, it could be concluded that this vegetables are natural sources of amino acids substances of high importance.

Key words: Amino acid · Bioactive · Vegetables · Metabolites and nutrients

INTRODUCTION

Amino acids are essential in the synthesis of proteins and precursors in the formation of secondary metabolism molecules [1] that participate in cell signaling, gene expression and homeostasis regulation [2], protein phosphorylation, synthesis of hormones and antioxidant capacity [3]. Also, amino acids participate in various physiological processes such as skeletal muscle function, atrophic conditions, sarcopenia and cancer [4]. Balance diet intake can provide our needs for energy and body building; all power is based on the principle of food metabolism for our well-being. Furthermore, the issue of food security in developing countries renders the population to consume a balanced diet by families made with dishes of basic food (cereal, tuber) rich in energy and sauce [5]. Different parts of plants are widely used as vegetables in the preparation of many dishes in many countries like Nigeria [6]. Essential amino acid (EAA) deficiency induces the slowdown of growth and development in children, gives diseases and causes the destruction of cells in adults [7]. Among these amino acids, some are bioactive (histidine, lysine, isoleucine, leucine, valine, threonine and phenylalanine + tyrosine) and other are antioxidants (histidine, methionine + cysteine) [8].

Medicinal plants are used in various parts of the world to alleviate many disease conditions and ailments [9]. It is in acknowledgement of this fact that research into medicinal plants are encouraged with the view to bringing back to the fore the past documented medicinal uses of herbs [10]. Amino acids, are often referred to as the building blocks of proteins, are compounds that play many critical roles in our body. They are needed for many

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vital processes in the body such as; the building of proteins and synthesis of hormones and neurotransmitters [11]. Some may also be taken in supplement form for a natural way to boost athletic performance or improve mood [12]. They are categorized as essential, conditionally essential or nonessential depending on several factors [13].

Pterocarpus santalinoides is a tree species in the legume family (Fabaceae); it is locally known as *uturukpa (*Ezzamgbo ohaukwu LGA Ebonyi State Nigeria). It has a remarkable bi-continental distribution, native to tropical western Africa (Benin, Burkina Faso, Cameroon, Gambia, Ghana, Guinea, [14].

Ficus sur is a fast-growing, deciduous or evergreen tree [15] also locally called akpuru. It usually grows from 5-12 metres (16-39 ft) in height, but may attain a height of 35-40 metres (115-131 ft). The large, alternate and spirally arranged leaves are ovate to elliptic with irregularly serrated margins [16]. Fresh foliage is a conspicuous red colour and the papery, 1 cm long stipules are soon dropped [17]. The bark of younger trees is smooth and pale greyish-white in colour [18].

The figs are carried on short or long droopingspurs which may emerge from surface roots, the trunk or especially from lower main branches [19]. The Figs are 2 to 4 cm in diameter and acquire a rosy, speckled exterior when ripe. The figs are edible and utilized in fresh or dried form by native people in Ezzamgbo and other regions. They are also suited to preparation of fig preserve, if other suitable fruit are added [20].

Vitex doniana is extremely widespread in tropical Africa, occurring from Senegal east to Somalia and south to South Africa; also in Comoros and Seychelles [21]. It is occasionally cultivated elsewhere, e.g. in Mauritius. The plant is often used in traditional medicine. Modern research has shown that the plant has a range of actions upon the body.

An aqueous extract of the stem bark has been shown to produce a dose-dependent hypotensive effect and to also be hepato-protective [22]. Stem bark extracts can inhibit the growth of clinical isolates of *Salmonella typhi, Shigella dysenteriae* and *Escherichia coli*, suggesting that they may be valuable in the treatment of dysentery and other gastroenteritic infections [23].

Wild food plants play an important role in the diet of inhabitants of Ezzamgbo in Ohaukwu Local government Ebonyi State Nigeria. Some of these plants are drought-resistant and gathered throughout the season. These foods are an important source of nutrients. However, there is a lack of comprehensive data regarding the nutrient content of these indigenous plants [24].

In a context of fighting against deficiencies in food, it is important to estimate the nature and amounts of micronutrients ingested by the people from traditional foods [25].

Hence, the purpose of this study was to investigate the amino acid content of *Pterocarpus santalinoides, Ficus sur* and *Vitex doniana* vegetable leaves indigenous in Ezzamgbo in Ohaukwu local government Ebonyi State Nigeria .

MATERIALS AND METHODS

Collection and Preparation of Plant Material: Fresh leaves of *Pterocarpus santalinoides and Vitex doniana*were collected from amovu-Amike Ezzamgbo in Ohaukwu local government Area Ebonyi State Eastern Nigeria. The plants were authenticated by a taxonomist at the Department of Biology, Ebonyi State University of Nigeria by comparison with voucher specimen deposited at the Department. The leaves were separated from the stem, washed and dried for two weeks at room temperature. The dried leaves of *Pterocarpus santalinoides, Ficus sur and Vitex doniana* were ground into powder using a manual blender and stored in a cool dry container until analysis.

PTEROCARPUS SANTALINOIDES

FICUS SUR

VITEX DONIANA

Methods

Crude Protein Determination: Crude protein content was determined by Mirokjedahi method as described by [26]. The micokjedahi method involves digestion, distillation and filtration.

Digestion: Small quantity of the *Pterocarpus santalinoides and Vitex doniana leaf* flour (0.1g) was weighed into kjedahi flask with 2.0g catalyst (sodium sulphate) respectively. This was followed by the addition of 20ml concentrated H_2SO_4. The flask and its content were gently heated. The heating was increased until the content of the flask was completely digested to give a clear solution.

Distillation: The content of the flask was then washed with 200ml distilled water separately into a distillation flask and cooled under ice block. This was followed by the addition of 100ml of 4% Boric acid poured into each of the flask and 3 drops of screened methyl red then added.

Titration: About 50ml of cooled 40% NaOH was added and the distillate was then titrated against 0.5n Na_2S0_4 Solution.

Determination of Amino Acid Profile: The amino acid profile in the samples were determined using methods described by [27]. The samples were dried to constant weight, defatted and hydrolyzed, evaporated in a rotary evaporator and loaded into the technicon sequential multi-sample Amino Acid analyzer (TSM).

Defeating of Sample: A2G of the dried sample was weighed into extraction thimble and the fat was extracted with chloroform/methanol (2:1 mixture) using soxhlet extraction apparatus as described by [28]. The extraction lasted for 15 hours.

Nitrogen Determination: A small amount (200mg) of ground sample was weighed, wrapped in whatman filter paper (No1) and put in the kjedahl digestion flask. Concentrated sulphuric acid (10ml) was added. Catalyst mixture (0.5g) 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 [29].

The flask was then put in Kjedahi digestion apparatus for 3 hours until the liquid turned light green. The digested samples were cooled and diluted with distilled water to 100ml in standard volumetric flask respectively. Aliquot (10ml) of the diluted solution with 10ml of 45% sodium hydroxide was put into the Markham distillation apparatus and distilled into 10ml of 2% boric acid containing 4 drops of bromocresol green/methyl red indicator until about 70ml of distillate was collected. The distillate was then titrated with standardize 0.01N hydrochloric acid to grey coloured end point, the percentage nitrogen in the original sample was calculated using the formula:

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

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: A 30-35mg of the defatted *Pterocarpus santalinoides Ficus sur and Vitex doniana*leaves were weighed into glass ampoule. 7ml of 6N 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 example methionine and cysteine). The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at 105± 5°C for 22 hours. The ampoule was allowed to cool before broken open at the tip and the content were filtered to remove the humans. It should be noted that tryptophan is destroyed by 6N HCl during hydrolysis.

The filtrates were then evaporated to dryness at 40°C under vacuum in a rotary evaporator. The residue was dissolved with 5ml of acetate buffer ($PH_{2.0}$) and stored in plastic specimen bottles which were kept in the freezer.

Loading of the Hydrolysate into TSM Analyzer: The amount loaded was between 5 to 10 micro litres respectively. These were dispensed into the cartridge of the analyzed free fatty acid. Neutral and basic amino acids of the hydrosylate. The period of an analysis lasted for 76 minutes.

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) were measured, the half-height of the peak on the charts were found and width of the peak on the half height accurately measured and recorded. Approximately area of each peak were then obtained by multiplying the height with the width at half-height.

The Norleucine Equivalent (NE) for each amino acid in the standard mixture was calculated using the formula:

$$NE = \frac{\text{Area of Norleucine Peak}}{\text{Area of each amino acid}}$$

A constant called S was calculated for each amino acid in the standard mixture as:

$$S_{std} = Ne_{std} \text{ x molecular weight x } \mu MAA_{std}$$

Finally, the amount of each amino acid present in each sample were calculated in g/16gN or g/100g protein using the following formula:

$$\text{Concentration (g/100g protein)} = NH^{x}WNH/12xS_{std}Xc$$

where:
C = Dilution x 16

Sample wt(g) x N% X10xvol. Loaded + NH x W(Nleu)

where:
NH = Net height
W = Width at half height
nLeu = Norleucine

Determination of Quality of Dietary Protein and Predicated Protein Efficiency Ratio (P-PER): Dietary protein was measured by finding the ratio of available amino acids in the protein concentrate compared with needs expressed as a ratio [30]. Amino acid score (AMSS) were then estimated by applying the [31] formula:

$$Amss = \frac{\text{mg of amino acid of 1g of test protein x 100}}{\text{Mg of amino acid of 1g reference protein}}$$

Table 1: Amino acid composition (g/100g crude protein) of *Pterocapus santalinoide*

AMINO ACID	*Pterocapus Santalinoide*
Leucine	8.29
Lysine	4.30
Isoleucine	3.86
Phenylalanine	4.26
Tryptophan	0.89
Valine	4.62
Methionine	1.23
Proline	3.04
Arginine	5.16
Tyrosine	2.92
Histidine	2.36
Cysteine	1.09
Alanine	3.87
Glutamic acid	10.60
Glycine	3.51
Threonine	2.89
Serine	2.32
Aspartic acid	9.05
Total	74.26

Table 2: Amino acid composition (g/100g crude protein) of *Ficus sur*

AMINO ACID	*Ficus Sur*
Leucine	7.59
Lysine	3.61
Isoleucine	3.99
Phenylalanine	3.55
Tryptophan	0.73
Valine	3.68
Methionine	0.91
Proline	2.23
Arginine	4.30
Tyrosine	3.27
Histidine	1.98
Cysteine	0.85
Alanine	4.02
Glutamic acid	8.78
Glycine	3.23
Threonine	3.94
Serine	1.89
Aspartic acid	8.31
Total	66.86

Table 3: Amino acid composition (g/100g crude protein) of *Vitex doniana*

AMINO ACID	*Vitex doniana*
Leucine	5.91
Lysine	5.37
Isoleucine	6.63
Phenylalanine	17.96
Tryptophan	5.32
Valine	5.92
Methionine	5.41
Proline	20.56
Arginine	17.42
Tyrosine	34.84
Histidine	6.47
Cysteine	12.26
Alanine	7.68
Glutamic acid	15.33
Glycine	4.81
Threonine	5.62
Serine	5.47
Aspartic acid	6.28
Total	189.26

Table 4: Classification of amino acid composition (g/100g crude protein) of *Pterocapus santalinoide*

AMINO ACID	*Pterocapus Santalinoide*
Total amino acid (TAA)	74.26
Total Non-essential amino acid (TNEAA)	37.29
PercentagE(%) TNEAA	50.22%
Total essential amino acid (TEAA)(with histidine)	36.97
TEAA (Without histidine)	34.61
Percentage TEAA(with histsidine)	49.78%
Percentage TEAA(without histsidine)	46.61 %
Essential aliphatic amino acid (EAA)	16.77
Essential aromatic amino acid (EArAA)	7.51
Total neutral amino acid (TNAA)	11.82
Percentage TNAA	15.92 %
Total acidic amino acid (TAAA)	19.65
Percentage (%) TAAA	26.46 %
Total basic amino acid (TBAA)	11.82
Percentage TBAA	15.92 %
Total Sulphur amino acid (TSAA)	2..32
Percentage of cysteine in TSAA	46.98

Table 5: Classification of amino acid composition (g/100g crude protein) of *Ficus sur*

AMINO ACID	*Ficus Sur*
Total amino acid (TAA)	66.86
Total Non-essential amino acid (TNEAA)	33.36
PercentagE(%) TNEAA	49.89 %
Total essential amino acid (TEAA)(with histidine)	33.5
TEAA (Without histidine)	31.52
Percentage TEAA(with histsidine)	50.10 %
Percentage TEAA(without histsidine)	47.14 %
Essential aliphatic amino acid (EAA)	21.51
Essential aromatic amino acid (EArAA)	6.26
Total neutral amino acid (TNAA)	9.89
Percentage TNAA	14.79 %
Total acidic amino acid (TAAA)	17.09
Percentage (%) TAAA	25.56 %
Total basic amino acid (TBAA)	9.89
Percentage TBAA	14.79 %
Total Sulphur amino acid (TSAA)	1.76
Percentage of cysteine in TSAA	48.30%

Table 6: Classification of amino acid composition (g/100g crude protein) of *VITEX DONIANA*

AMINO ACID	*Vitex Doniana*
Total amino acid (TAA)	189.26
Total Non-essential amino acid (TNEAA)	112.55
PercentagE(%) TNEAA	59.46 %
Total essential amino acid (TEAA)(with histidine)	76.71
TEAA (Without histidine)	70.24
Percentage TEAA(with histsidine)	40.53 %
Percentage TEAA(without histsidine)	37.11 %
Essential aliphatic amino acid (EAA)	46.7
Essential aromatic amino acid (EArAA)	29.26
Total neutral amino acid (TNAA)	29.26
Percentage TNAA	15.46 %
Total acidic amino acid (TAAA)	21.61
Percentage (%) TAAA	11.42 %
Total basic amino acid (TBAA)	29.26
Percentage TBAA	15.46 %
Total Sulphur amino acid (TSAA)	1.76
Percentage of cysteine in TSAA	48.30%

The predicted protein efficiency ratio (P-PER) of the vegetable samples were calculated from their amino acid composition based on the equation developed by [32] as stated thus: P- PER = - 0.468+ 0.454 (Leu)-0.015 (Tyr).

DISCUSSION

Amino acids are building blocks of proteins. They are bifunctional compounds containing both an amine group and a carboxylic acid group [33]. In other words, they are derivatives of the carboxylic acids in which an amino group replaces a hydrogen atom in the carbon chain [34]. Most amino acids occurring naturally in proteins are of the α type, having the amino group attached to the carbon atom adjacent to the carboxyl group [35]. Proline which has an imino (NH) group instead of an amino group. The nature of the 'R' group, which is referred to as the side-chain, varies in different amino acids. It may simply be a hydrogen atom, as in glycine, or it may be a more complex radical having, for example, a phenyl group [35]. There are nine essential (indispensable) amino acids, which cannot be synthesized in the body [36] and if one of these is provided in inadequate amounts regardless of the total protein intake, it will not be possible to maintain nitrogen balance [37]. Two amino acids, cysteine and tyrosine, can be synthesised in the body only from essential amino acids precursors - cysteine from methionine and tyrosine from phenylalanine [37]. The non-essential (dispensable) amino acids are the ones that can be synthesised from metabolic intermediates, as long as there is enough total protein in the diet [38].

In this study the evaluation of amino acid composition of three indigenous vegetables in Ohaukwu LG. Area in Ebonyi State Nigeria were carried out. The results showed the presence of both essential and non essential amino acids in varying concentrations in the vegetables many of which play several important roles in our body [39]. However, among the essential amino acids include: Leucine a branched-chain amino acid that is critical for protein synthesis and muscle repair were significantly ($p > 0.05$) in all the vegetables. This amino acid specifically helps regulate blood sugar levels, stimulates wound healing and produces growth hormones [40] (Table 1, 2 and 3). While lysine has been reported to plays major roles in protein synthesis, hormone, enzyme production and the absorption of calcium. It is also important for energy production, immune function and the production of collagen and elastin [41]. Furthermore isoleucine is involved in muscle metabolism and is heavily

concentrated in muscle tissue. It is also important for immune function, hemoglobin production and energy regulation [42].

Phenylalanine in this study showed an appreciable amount in the vegetables investigated (Table1, 2 and 3). It is a precursor for the neurotransmitters tyrosine, dopamine, epinephrine and norepinephrine. It plays an integral role in the structure and function of proteins and enzymes and the production of other amino acids [43]. However, tryptophan were also present and has many functions. It is needed to maintain proper nitrogen balance and is a precursor to serotonin, a neurotransmitter that regulates our appetite, sleep and mood [44]. Valine is one of three branched-chain amino acids, which implies that it has a chain branching off to one side of its molecular structure. Valine helps stimulate muscle growth and regeneration and is involved in energy production [45].

Whereas methionine plays an important role in metabolism and detoxification. It is also necessary for tissue growth and the absorption of zinc and selenium, minerals that are vital to our health [46].

Other essential amino acid includes histidine and threonine. Histidine is used to produce histamine, a neurotransmitter that is vital to immune response, digestion, sexual function and sleep-wake cycles. It is critical for maintaining the myelin sheath, a protective barrier that surrounds the nerve cells [47]. While threonine is a principal part of structural proteins such as collagen and elastin, which are important components of the skin and connective tissue. It also plays a role in fat metabolism and immune function [48].Thus consumption of these plants may ameliorate skin and connective tissue related diseases .

On the other hand non-essential amino acids evaluated in this work play several important roles in our body. Some of which include alanine, an important source of energy for muscle tissue, brain and the central nervous system. It strengthens the immune system by producing antibodies, also helps in the metabolism of sugars and organic acids whereas glycine facilitates the release of oxygen to cell for energy generation [49]. It aid in the manufacture of hormones responsible for a strong immune system [50].

Another non-essential amino acid Arginine, were also found in appreciable amount in all the vegetables. Reports have shown that it improves immune responses to bacteria, viruses and cancer cells, promotes wound healing and regeneration of the liver; causes the release of growth hormones and is crucial for optimal muscle growth and tissue repair [51], necessary for production of the amino acid Ornithine. Glutamic acid is one of the non-essential amino acid which was present in both these leaves in fairly large amounts. It was found to be significantly ($p > 0.05$) high in these plants (Table 1, 2 and 3) and they are present in many foods and are considered protective against obesity and cardiovascular diseases [52]. Along with essential amino acids, the body can incorporate them into new proteins as the cells need them. In addition, depending on diet intake, they can undergo chemical conversion to ultimately create glucose, for use as a fuel source, or fatty acids, for storage of excess calories [53]. However, the characteristic unique to non-essential amino acids is their synthesis from other biological sources within the cells, when the diet does not provide enough of them. In contrast, our body cannot manufacture essential amino acids, which is reason people must include them in the foods they eat [54].

The results of total amino acid, non essential amino acid, essential amino acid, essential aliphatic amino acids, acidic amino, essential aromatic amino acid, total acid and basic amino acids were significantly ($p > 0.05$) high .However, in this study the amino acid composition (g/100g crude protein) were classified as shown on Table 4, 5 and 6. These amino acids play vital roles in metabolism to maintain balance in protein requirements. Thus a balance between amino acids appears to be important for normal physiological functions and this will enhance well being of both omnivores and herbivores.

Furthermore, this study has shown that the vegetables investigated are potential sources of nutrients that will enhance food security and may reduce risks associated with nutrients deficiency diseases.

CONCLUSION

The present study clearly indicates that the leaves of *Pterocarpus santalinoides, Ficus sur* and *Vitex doniana* posses good amount of amino acid that make up the proteins in them. Thus their composition suggests that these plants can serve as a rich source to meet out the amino acid requirements of human and animals based on the fact that all the major essential amino acids were found to be present in all the vegetables investigated.

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