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Gas chromatography analysis of the amino acid composition of proteins from the leaves of *Acalypha wilkesiana* Muell Arg and *Tridax procumbens* Linn

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ABSTRACT

The amino acid composition of proteins from leaves of *Acalypha wilkesiana* and *Tridax procumbens* was studied using gas chromatography. *A. wilkesiana* leaf protein had lower total essential, but higher total sulphur containing and aromatic amino acid content than *T. procumbens* leaf protein. Both proteins had high contents of essential amino acids, histidine, threonine and valine, though deficient in isoleucine. Thus both proteins may serves as good sources for the supplementation of histidine, threonine and valine.

Introduction

Acalypha wilkesiana and *Tridax procumbens* are medicinal plants commonly used in Southern Nigeria. *Acalypha wilkesiana* Muell Arg belongs to the family Euphorbiaceae, and is commonly called copperleaf, Joseph's coat, fire dragon and beef steak plant [1, 2]; while *Tridax procumbens* Linn belongs to the family Asteraceae (alt. Compositae) and is commonly called coat buttons, wild daisy and tridax. Both plants have anti-anaemic [3, 4], anti-diabetic [5, 6, 7], antihypertensive [8, 9, 10, 11], antimicrobial [12, 13, 14, 15, 16], hepatoprotective [17, 18, 19, 20], weight reducing and hypocholesterolaemic [21, 22, 23] properties. In addition, *T. procumbens* is also used in the management of bronchial catarrh, malaria, stomach ache, diarrhoea, haemorrhage from cuts, bruises and wounds and hair loss. It has anti-inflammatory, antioxidant [17], anti-arthritic [24], and analgesic [25] properties, and marked depressant action on respiration [17, 26, 27, 28]. *Acalypha wilkesiana* has diuretic activity [29]. The leaves of *T. procumbens* is used as a fodder and is also eaten as vegetable in India [28, 30], while some herbal practitioners in Southern Nigeria prescribe the chewing of raw *A. wilkesiana* leaves for the management of hypertension. Consequent upon this, their proximate, mineral, vitamin and phytochemical compositions were investigated [31, 32, 33, 34, 35]. So, in the present study the amino acid profiles of proteins from the leaves of *A. wilkesiana* and *T. procumbens* was investigated; a prelude to assessing their likely contribution to the nitrogen balance of their consumers.

Materials and Methods

Collection of plant samples and reagents

Samples of fresh *Acalypha wilkesiana* and *Tridax procumbens* were collected from within the Abuja and Choba Campuses of University of Port Harcourt, Port Harcourt, Nigeria. They were identified by comparison to samples earlier identified by Ikewuchi [20] and Ikewuchi *et al*. [7]. Their leaves were removed, rid of dirt, oven dried at 55 °C and ground into powder. The resultant powder was stored in air-tight containers for subsequent use. All reagents used were of analytical grade purity.

Determination of the amino acid composition

The amino acid profile of each sample was determined using method described by Spackman *et al*. [36]. The samples were dried to constant weight, defatted, hydrolyzed, concentrated in a rotary evaporator and 10 µL of the hydrolysate was loaded into the gas chromatogram of model HP6890, powered with HP Chemstation Rev A09.01(1206) software, with pulse flame photometric detector. The capillary was a ZP-5 column (30 m × 0.32 mm ID × 0.25 µm film thickness). The oven temperature was held at 50 °C for 2 min, and then ramped at 10 °C/min for 4 min before changing to 15 °C/min for 5 min. Injection was at a split ratio of 1:20. Nitrogen was used as carrier gas. The column and compressed air pressures were respectively 20 and 30 psi. The injector and detector temperatures were 250 and 320 °C, respectively.

Determination of digestible indispensable amino acid (DIAA) reference ratio and DIAA score

The digestible indispensable amino acid reference ratio for each indispensable amino acid (IAA) in the test proteins was determined by comparing their amino acid composition, with WHO reference protein patterns [37], as follows:

$$\text{DIAA reference ratio} = \frac{A}{B}$$

Where A = mg of a DIAA in 1 g of the test protein; B = mg of the same DIAA in 1 g of the reference protein

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The lowest DIAA reference ratio obtained herein was converted to percentage and used as the digestible IAA score (DIAAS); while the DIAA with the least ratio was taken as the limiting DIAA [37].

Derivation of compositions per wet weight

The compositions per wet weight of the determined parameters were derived from their compositions per dry weight, using the following formula [38].

$$\textbf{Composition per wet weight}\ (\%) = \frac{\%\ \textbf{composition per dry weight} \times \%\ \textbf{dry matter content}}{\mathbf{100}}$$

Data analysis

Comparisons were based on simple percentages.

Results and Discussion

The amino acid profiles and chemical scores of proteins from the leaves of *A. wilkesiana* and *T. procumbens* are given in Tables 1 and 2 respectively. The *A. wilkesiana* leaf protein has lower total essential (about 56.60%), but higher total sulphur containing (4.34%) and aromatic amino acid (7.17%) content than *T. procumbens* leaf protein (67.51%, 3.49% and 4.77% respectively). Every 1 g of the protein from *A. wilkesiana* contains 320.454 mg of essential amino acids, 24.560 mg of sulphur-containing amino acids and 40.578 mg of aromatic amino acids (Table 1), while that of *T. procumbens* contains 410.286 mg of essential amino acids, 21.180 mg of sulphur-containing amino acids and 28.978 mg of aromatic amino acids. Both proteins were rich in the essential amino acids, histidine, threonine and valine, and can meet their minimum daily requirements [37]. In comparison to the WHO reference protein patterns [37], their limiting amino acid was isoleucine, with digestible IAA scores of 0, with reference to infant (birth to 6 months), child (6 months to 3 year) and older child, adolescent, adult protein requirement (Table 2). The implication of this is that if consumed, there must be supplementation of isoleucine, either through fortification or augmentation by combining them with another protein source that is rich in isoleucine. In comparison to child (6 months to 3 year) requirement protein pattern [37], their DIAAS was less than those reported for cooked peas (57.9%), cooked kidney beans (58.8%), cooked rice (59.5%), cooked rolled oats (54.2%), wheat bran (41.1%), roasted peanuts (43.4%), and rice protein concentrate (RPC) (37.1%) [39].

Table 1. Amino acid profile of proteins from the leaves of *Acalypha wilkesiana* and *Tridax procumbens*

Parameters	Composition					
	Acalypha wilkesiana			*Tridax procumbens*		
	mg/g protein	mg/100 g sample		mg/g protein	mg/100 g sample	
		/fresh weight	/dry weight		/fresh weight	/dry weight
Lysine*	24.024	80.876	190.746	137.293	87.91	877.300
Histidine*	57.028	191.989	452.804	38.860	24.708	248.318
Arginine	20.872	70.267	165.723	13.832	8.794	88.386
Aspartate	59.543	200.456	472.774	39.459	25.089	252.146
Threonine*	85.567	288.066	679.402	92.728	58.957	592.531
Serine	37.029	124.660	294.009	24.539	15.602	156.805
Glutamate	39.513	133.023	313.733	26.185	16.649	167.324
Proline	10.991	37.002	87.269	7.284	4.631	46.544
Glycine	0.000	0.000	0.000	32.987	20.973	210.787
Alanine	43.955	147.976	348.999	29.129	18.520	186.133
Cystine*	16.312	54.916	129.520	14.980	9.524	95.719
Valine*	90.004	303.002	714.628	87.532	55.653	559.330
Methionine*	8.247	27.765	65.484	6.201	3.942	39.622
Isoleucine*	0.000	0.000	0.000	0.000	0.000	0.000
Leucine*	32.473	109.325	257.838	27.720	17.625	177.132
Tyrosine	17.467	58.802	138.685	9.026	5.739	57.676
Phenylalanine*	23.111	77.805	183.503	19.952	12.686	127.493
Total essential amino acids	320.454	1078.830	2544.406	410.286	260.862	2621.726
Total nonessential amino acids	245.682	827.102	1950.713	197.421	125.521	1261.52
Total sulphur-containing amino acids	24.560	82.682	195.004	21.180	13.466	135.341
Total aromatic amino acids	40.578	136.608	322.188	28.978	18.424	185.169

*Essential amino acids

Table 2. Comparison of *Acalypha wilkesiana* and *Tridax procumbens* leaf proteins with WHO reference protein pattern

Amino acids	Reference patterns (mg/g protein)			DIAA reference ratio (%)					
				Acalypha wilkesiana			*Tridax procumbens*		
	Infant (birth to 6 months)	Child (6 months to 3 year)	Older child, adolescent, adult	A	B	C	A	B	C
Histidine	21	20	16	2.716	2.851	3.564	1.851	1.943	2.429
Isoleucine	55	32	30	0.000	0.000	0.000	0.000	0.000	0.000
Leucine	96	66	61	0.338	0.492	0.532	0.289	0.420	0.454
Lysine	69	57	48	0.348	0.422	0.501	1.990	2.409	2.860
Methionine + cystine	33	27	23	0.744	0.910	1.068	0.642	0.784	0.921
Phenylalanine + tyrosine	94	52	41	0.432	0.780	0.990	0.308	0.557	0.707
Threonine	44	31	25	1.945	2.760	3.423	2.108	2.991	3.709
Valine	55	43	40	1.636	2.093	2.250	1.592	2.036	2.188

A = Comparison to Infant (birth to 6 months) requirement protein pattern; B = Comparison to Child (6 months to 3 year) requirement protein pattern; C = Comparison to Older child, adolescent, adult requirement protein pattern

Although *T. procumbens* has a comparatively higher total essential amino acid content, *A. wilkesiana* was found to have higher histidine, leucine, valine, aromatic and sulphur containing amino acid content, but with lower lysine and threonine. Therefore both proteins can serve as good sources of histidine, leucine, valine (and lysine, in the case of *T. procumbens* only), especially for nutritional supplementation in cases of the deficiency of the aforementioned amino acids.

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