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NUTRIENT AND BIOACTIVE PHYTOCHEMICAL COMPOSITIONS OF *Cnidoscolus aconitifolius*

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Abstract

Cnidoscolus aconitifolius is consumed as a vegetable in many parts of Southern Nigeria. Presently, there is no information regarding the allicins alkaloids, carotenoids, glycosides, phytosterols, saponins and terpenoids profiles of the leaves and stems of this vegetable. The proximate, minerals, vitamins, amino acid, carotenoids, saponins, glycosides, phytosterols, terpenoids, alkaloids and allicins profiles of the leaves and stems of *Cnidoscolus aconitifolius* were determined using standard methods. The leaves and stems had high fibre (14.0 - 15.2 g/100g), carbohydrate (40.2 - 41.2 g/100g) and protein (22.2 - 24.5 g/100g). These were equivalent to about 56.0-66.9% daily value for fibre, 44.4-49.0% daily value protein and 13.4-15.1% daily value carbohydrate. They had high contents of selenium, copper, manganese, iron, cobalt, magnesium (leaves only) and vitamins E, B9, C and K (stems only), carotenoids, saponins and glycosides; and moderate phytosterols and terpenoids. Their proteins were rich in essential amino acids (42.7-45.6%). Ten known carorenoids were detected in the stems and leaves, consisting mainly of carotene (43.7-46.1%), lutein (20.8-22.5%) and neo-xanthin (10.92-12.99%). Sapogenin (62.99-64.56%) and saponine (24.5-28. 9%) were the most abundant of the eleven saponins detected. Of the twelve known glycosides were detected, artemetin (65.8-67.6%) and digitoxin (24.8-27.7%) were the most abundant. Seven phytosterols were detected, and consisted mainly of sitosterol (63.6-71.3%), stigmasterol (10.9-13.6%) and 5-avenasterol (10.6-11.4%). Beta-amyrin (52.2-66.3%), alpha-amyrin (18.5-31.6%) and lupeol (14.8-15.9%) were the most abundant of the five phytosterols detected. This result indicates that the leaves and stems of *Cnidoscolus aconitifolius* are good sources of nutrients and bioactive phytochenicals that can support human health and nutrition. This rich profile makes them potential sources of nutraceuticals.

INTRODUCTION

Cnidoscolus aconitifolius (Euphorbiaceae), commonly called "hospital too far", "treadsoftly" or tree spinach (English), "Chaya" (Mexico), "Efo Iyana Ipaja" or "Efo Jerusalem" (Yoruba), is consumed as a vegetable in many parts of Southern Nigeria [1, 2]. Its shoot and leaves are employed traditionally to enhance memory, stimulate lactation, strengthen fingernail, darken grey hair, as well as treat acne, alcoholism, anaemia, diabetes, eye problems, fever, gastrointestinal disorders, hypertension, inflammation, insomnia, kidney stones, malaria, nasal congestion, obesity, scorpion stings and varicose veins [1, 2, 3, 4, 5].

The leaves contain bioactive principles such as anthraquinones, cardiac glycosides, phenols, flavonoids, phlobatannin and triterpenoids saponins [5, 6]. GC-MS profiling of the leaf extracts revealed the presence of octadecenoic acid and its esters, n-hexadecanoic acid, n-octadecanoic acid, n-octacosane, 1,2,3-propanetriol and its derivatives, ascorbic acid-2,6-dihexadecanoate, borneol, caryophyllene oxide, 4-(1,5-dimethyl hex-4-enyl) cyclohex-2-enone, farnesol and longipinane [1, 5]. Presently, there is no information regarding the allicins alkaloids, carotenoids, glycosides, phytosterols, saponins and terpenoids profiles of the leaves and stems of this vegetable. Therefore, this study investigated the profiles of these compounds in the leaves and stems of *Cnidoscolus aconitifolius*, as well as their nutrient profiles; with a view to providing information on their potential as sources of nutrients and nutraceuticals. It is also intended to provide an overview of their potential contributions to the human diet, as well as the pharmacological significance of some of the bioactive constituents.

MATERIALS AND METHODS

Materials

Fresh samples of the leaves and stems of *Cnidoscolus aconitifolius* were collected from a house garden in Aluu community in Rivers State, Nigeria. They were identified (voucher number: UPH/V/1332) by Dr. Chimezie Ekeke at the Herbarium of the Department of Plant Science and Biotechnology, University of Port Harcourt, Port Harcourt, Nigeria. The chemicals used for the assays were of analytical grade and procured from Sigma-Aldrich Co. and Lynnchem Biological Technology Co. The atomic absorption spectrophotometer was a SensAA (GBC Scientific Equipment, USA); while the gas chromatograph was a Hewlett Packard HP 6890 (fitted with flame ionization detector or pulse flame photometric detector) powered with HP Chemstation Rev. A09.01 (1206) software.

Methods

Proximate analysis

The proximate components were determined in triplicates. The moisture content was determined by AOAC Official Method 967.03 [7], ash by AOAC Official Method 942.05 [7], total lipid by AOAC Official Method 920.39 [7], fibre by AOAC Official Method 973.18 [7], and crude protein (% total nitrogen x 6.25) by AOAC Official Method 2001.11 [7]. Carbohydrate was determined by difference (i.e. by subtracting the sum of all the other components from 100 g). The caloric values were calculated with the Atwater factors 4, 9 and 4 for protein, fat and carbohydrate respectively [8].

Determination of vitamin profile

The vitamin profiles were analysed by a combination of AOAC Official Methods 992.03, 992.04 and 992.26 [7]. Chromatographic conditions were similar to that reported by Ikewuchi *et al*. [8], except for the use of HP 5 column, and compressed air pressure of 241.32 kPa.

Determination of mineral elements and phosphorus composition

Analysis of the mineral elements was carried out according to FAO fertilizer and plant nutrition bulletin 19 [9]. Phosphorus was determined by vanadium molybdate method [9].

Determination of per cent daily value

By comparing to daily values [10], per cent daily values were calculated, as follows:

$$Percent\ daily\ value\ (\%) = \frac{weight\ of\ particular\ nutrient\ in\ 100g\ of\ sample}{daily\ value} \times 100$$

Amino acid analysis

The extraction and analysis were carried out following the methods of AOAC Method 982.30(a,b,c) [7] and Obreshkova *et al*. [11]. The gas chromatograph was fitted with a pulse flame photometric detector. A split injection (split ratio: 20:1) was adopted, with hydrogen as carrier gas, at flow rate of 1.0 mL/min. An EZ column (10 m x 0.2 mm i.d. x 0.25 µm film thickness), was used. The inlet and detector temperatures were 250 and 320 °C. The hydrogen and compressed air pressure were 137.90 and 241.32 kPa. The oven was programmed initially at 110 °C, ramped at 7 °C/min to 320 °C; and kept at 320 °C for 5 min.

Evaluating digestible indispensable amino acid (DIAA) reference ratio and DIAA score

The digestible indispensable amino acid (DIAA) reference ratio for each indispensable amino acid (IAA) in the test proteins were determined by comparing their amino acid composition, with WHO reference protein patterns [12], according to the following equation.

$$\text{DIAA}\ reference\ ratio = \frac{mg\ of\ a\ \text{DIAA}\ in\ 1g\ protein\ of\ the\ sample}{mg\ of\ the\ same\ \text{DIAA}\ in\ 1g\ of\ reference\ protein}$$

The DIAA with the least DIAA reference ratio became the limiting amino acid; while its ratio was converted to percentage, to obtain the digestible IAA score (DIAAS) [12].

Determination of the phytochemical constituents

General procedures

The preparation of the standard solutions, as well as the identification and quantification of the component compounds were as earlier reported by Ikewuchi *et al*. [8].

Determination of alkaloids, allicins, carotenoids, glycosides and saponins compositions

The alkaloids were extracted in accordance with Ngounou *et al*. [13]; allicins according to Chehregani *et al*. [14]; carotenoids according to Takagi [15]; glycosides according to Oluwaniyi and Ibiyemi [16]; and saponins in line with Guo *et al*. [17]. The extracts were subjected to gas chromatography under similar conditions as reported by Ikewuchi *et al*. [8].

Determination of sterols composition

Extraction of oil was carried out according to AOAC Official method 999.02 [7], while analysis of sterols was carried out according to AOAC Official methods 994.10 and 970.51 [7]. The sterol fraction was then subjected to gas chromatography with similar conditions as reported by Ifeanacho *et al*. [18].

Derivation of compositions per dry weight from the composition per wet weight

Compositions per dry weight of the parameters were derived from compositions per wet weight and vice versa, using the following formula [19].

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

RESULTS AND DISCUSSION

The proximate composition and nutrient potential of the leaves and stems of *Cnidoscolus aconitifolius* is shown in **Table 1**. They had high protein and fibre, and moderate carbohydrate contents. In comparison to relevant daily values [10], a 100g serving of the leaves can provide about 56.0-60.3% of daily value for crude fibre, 13.4-14.4% of daily value for carbohydrate, 15.0-16.1% of daily value for caloric value, 6.9-7.5% of daily value for total lipid and 49.0-52.8% of daily value for crude protein. A 100g serving

of the stems can provide 60.8-66.9% of daily value for crude fibre, 60.8-66.9% of daily value for carbohydrate, 14.3-15.7% of daily value for caloric value, 5.4-5.9% of daily value for total lipid and 44.4-48.8% of daily value for crude protein.

Table 1. Proximate composition and nutrient potential of the leaves and stems of *Cnidoscolus aconitifolius*

Component	Composition (g/100g)‡				Potential (per cent daily value/100g)			
	Leaf		Stem		Leaf		Stem	
	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry
Moisture	7.20	0.00	9.10	0.00	NA	NA	NA	NA
Dry matter	92.80	100.00	90.90	100.00	NA	NA	NA	NA
Ash	9.60	10.34	8.80	9.68	NA	NA	NA	NA
Crude protein	24.50	26.40	22.20	24.42	49.00	52.80	44.40	48.85
Crude fat	4.50	4.85	3.50	3.85	6.92	7.46	5.39	5.92
Crude fibre	14.00	15.09	15.20	16.72	56.00	60.35	60.80	66.89
Total carbohydrate	40.20	43.32	41.20	45.32	13.40	14.44	13.73	15.11
Caloric value	299.30	322.52	285.10	313.64	14.97	16.13	14.26	15.68

Values are means of triplicate determinations. ‡The unit of caloric value = kcal/100g; NA = not applicable

The leaves and stems had low moisture levels. This is good for their "keeping" quality, because high moisture increases water activity and probability of microbial growth [20]. They had higher proteins than amaranth, cabbage, green and red lettuce [21, 22, 23, 24], *Pandiaka heudelotii* [8], *Tridax procumbens* [25], barley, maize and wheat, and are comparable to bean [26, 27]. They can be considered to be good protein sources, since they have greater proteins than the WHO 10% cut-off [12]. They had higher lipid, carbohydrate, ash and caloric value than *T. procumbens* [25], amaranth, cabbage, green and red lettuce [21 - 24]. They also had higher fibre than *P. heudelotii* [8], *T. procumbens* [25], cabbage, green and red lettuce [21 - 23]. Therefore, consuming them amounts to high fibre intake, which has been reported to enhance bowel clearance and modulate blood cholesterol, glucose and insulin responses [28].

Table 2. Mineral elements composition and nutrient potential of leaves and stems of *Cnidoscolus aconitifolius*

Mineral nutrient	Composition (mg/kg)				Potential (% daily value/100g)			
	Leaf		Stem		Leaf		Stem	
	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry
Sodium	366.73	395.18	1347.80	1482.72	1.53	1.65	5.62	6.18
Calcium	2461.65	2652.64	1963.09	2159.62	24.62	26.53	19.63	21.60
Magnesium	4827.22	5201.74	824.19	906.70	120.68	130.04	20.61	22.67
Potassium	6975.55	7516.76	3585.55	3944.50	19.93	21.48	10.24	11.27
Phosphorus	984.35	1060.72	1436.79	1580.62	9.84	10.61	14.37	15.81
Selenium	0.16	0.18	0.19	0.21	23457.10	25277.10	26757.10	29435.80
Cobalt	0.01	0.02	0.10	0.11	23333.33	25000.00	160000.00	176666.67
Copper	14.48	15.60	12.58	13.83	72.38	78.00	62.87	69.17
Manganese	17.43	18.78	9.46	10.41	87.14	93.90	47.32	52.06
Iron	92.16	99.31	86.90	95.60	51.20	55.17	48.28	53.11
Sodium/ potassium ratio‡	0.05	0.05	0.38	0.38	NA	NA	NA	NA
Calcium/ phosphorus ratio‡	2.50	2.50	1.37	1.37	NA	NA	NA	NA

The leaves and stems of *C. aconitifolius* had high copper, iron, manganese, selenium and magnesium (moderate in stems) (**Table 2**). A comparison to relevant daily values [10], shows that a 100g serving of the leaves and stems are respectively equivalent to 87.1 - 93.9% and 47.3-52.1% daily value for manganese; 23457.1-25277.1% and 26757.1-29435.8% daily value for selenium; and 72.4-78.0% and 62.9-69.2% daily value for copper. It is also equivalent to 120.7-130.0% and 20.6-22.7% daily value for magnesium; 51.2-55.2% and 48.3-53.1% daily value for iron; 24.6-26.5% and 19.6-21.6% daily value for calcium; 19.9-21.5% and 10.2-11.3% daily value for potassium; 9.8-10.6% and 14.4-15.8% daily value for phosphorus; and 1.5-1.7% and 5.2-6.2% daily value for calcium.

The leaves and stems had higher calcium, copper, iron, magnesium, manganese, phosphorus and selenium than amaranth, cabbage, green and red lettuce [21 - 24]. According to Korkmaz *et al*. [29], magnesium is a calcium channel blocker, and is involved in many different metabolic processes, including maintenance of cell membrane function, modulation of smooth muscle contraction and enzymatic activities. They further stated that magnesium is a neuroprotective agent; increases blood flow to tissues; plays a vital role in development and function of the eye; and in diabetic patients, decreases insulin resistance, enhances glycaemic control and prevents diabetic retinopathy [29]. Manganese functions both as a cofactor and activator to a large number of manganese-dependent enzymes, including arginase, decarboxylases, dehydrogenases, DNA and RNA polymerases, glutamine synthetase, kinases, manganese superoxide dismutase, neurotransmitter synthetic enzymes, oxidases and sugar transferases [30, 31, 32]. It is an integral part of certain metalloproteins [30, 32]; participates in the metabolism of biogenic amines and regulation of carbohydrate metabolism [30, 33). The leaves and stems had high calcium to phosphorus ratios. High dietary calcium/phosphorus ratio has a positive influence on bone mass [34]; and allows for strong bone development because absorption of calcium under this condition is maximal [35, 36].

The leaves and stems had higher potassium than *T. procumbens* [37], amaranth (leaves only), cabbage, green and red lettuce [21 - 24]. They had low sodium to potassium ratios ($\leq$ 1.67), and so, may be very safe for consumption by hypertensive individuals [38].

The leaves and stems had high vitamins E, B9, C and K (stems only) levels (**Table 3**). They had lower vitamins A and B3 than amaranth leaves, cabbage, green and red lettuce [21 - 24]. The vitamin B6 content of the stems was lower than that of amaranth [24], but higher than *T. procumbens* [39], cabbage, green and red lettuce [21 - 23]; while that of the leaves was lower than all of them. The leaves and stems had higher vitamins B2 and C than *T. procumbens* [39], amaranth, cabbage, green and red lettuce [21 - 24, 40]. They can respectively provide, per 100g serving, 76.3% and 100.3% of the daily value for vitamin C; as well as 14.9% and 18.5% of daily value for vitamin B2. The vitamin B1 content of the leaves was higher than *T. procumbens* [39], amaranth, cabbage, green and red lettuce [21 - 24]; while that of the stems was lower. The leaves can provide about 29.7% of the daily value for vitamin B1, per 100g serving. The stems had higher vitamins B5 and E than cabbage, green and red lettuce [21 - 23]; while leaves had lower values. They can respectively provide, per 100g serving, 141.0% and 7234.9% of daily value for vitamin E. Vitamin E is an anticoagulant, antiviral, immune-modulatory and neuroprotective agent [41].

The leaves and stems had higher vitamin B9 than *T. procumbens* [39], amaranth (leaves only), cabbage, green and red lettuce [21 - 24]. They can both provide 119.5% and 10.9% of the daily value for vitamin B9, respectively per 100g serving. The vitamin K content of the stems was higher than those of cabbage, but lower than those of amaranth, green and red lettuce [21 - 24]. That of the leaves was lower than all of them. They can respectively provide 13.9% and 155.8% of the daily value for vitamin K, per 100g serving.

Table 3. Vitamins composition and potential of the leaves and stems of *Cnidoscolus aconitifolius*

	Leaves			Stems		
	Retention time (min)	Composition (mg/kg)	Per cent daily value/100 g	Retention time (min)	Composition (mg/kg)	Per cent daily value/ 100 g
Vit. B3	12.366	0.802	0.401	12.373	2.282	1.141
Vit. B6	13.744	0.004	0.018	13.742	1.763	8.815
Vit. C	16.044	457.965	76.328	16.036	601.631	100.272
Vit. A	17.368	0.217	1.445	17.365	0.220	1.467
Vit. B1	18.060	4.455	29.697	18.053	0.051	0.340
Vit. B2	18.596	2.532	14.893	18.590	3.152	18.541
Vit. E	19.526	0.127	141.023	19.518	6.512	7234.870
Vit. B9	20.542	4.779	119.470	20.536	0.435	10.868
Vit. K	21.825	0.112	13.947	21.820	1.246	155.810
Vit. B5	22.606	0.037	0.037	22.600	2.791	2.791

The amino acid profile and DIAA reference ratios of proteins from the leaves and stems of *C. aconitifolius* are given in **Tables 4 and 5**, respectively. They are rich in essential amino acids, 45.6% for the leaves and 42.7% for the stems [especially histidine, valine, threonine, isoleucine, phenylalanine and tyrosine, lysine (leaves only) and tryptophan (stems only)] and can meet the daily requirements [12] for these essential amino acids. Compared to WHO reference protein patterns for infant (birth to 6 months), child (6 months to 3 years) and older child, adolescent, adult [12], the DIAA scores of the leaf protein were 46.60 (with tryptophan as the limiting amino acid), 75.07 and 88.13 (with methionine and cysteine as the limiting amino acid) respectively. Those of the stem protein were 49.38, 59.78 and 70.99, with lysine as the limiting amino acid. Every 100 g of these leaf and stems proteins contained, respectively, 42.54 g and 36.79 g of essential amino acids, 2.03 g and 2.12 g of sulphur-containing amino acids and 7.86 g and 8.26 g of aromatic amino acids (**Table 6**). The leaf and stem proteins can be used for the supplementation of histidine, valine, threonine, isoleucine, phenylalanine and tyrosine in all the age groups; tryptophan and lysine in the diets of children (6 months and above), adolescents and adults. Compared to child (6 months to 3 years) requirement protein pattern [12], proteins from the leaves and stems of *C. aconitifolius* had higher DIAA scores, than cooked peas, cooked kidney beans, cooked rice, cooked rolled oats, wheat bran, roasted peanuts and rice protein concentrate [42].

The leaves and stems had high carotenoids, saponins and glycosides, moderate phytosterols and terpenoids, and low allicins and alkaloids. Ten known carorenoids were detected in them (**Table 6**), made up of carotene (leaves: 46.12%; stems: 43.70%), lutein (leaves: 22.45%; stems: 20.75%), neoxanthin (leaves: 10.92%; stems: 12.99%), xanthophyll (leaves: 7.91%; stems: 7.48%), violaxanthin (leaves: 6.13%; stems: 6.27%), antheraxanthin (leaves: 4.91%; stems: 7.26%), astaxanthin (leaves: 1.53; stems: 1.55%). The other compounds made up less than 0.01%. The leaves and stems had higher carotene and lutein than *T. procumbens* [19], cabbage, green and red lettuce [21 - 23]. They had higher anthera-xanthin, neoxanthin and viola-xanthin than *T. procumbens* [19]. Their lycopene and malvidin contents were higher than cabbage, green and red lettuce [21 - 23].

Carotenes possess anticancer, antioxidant, immune-modulatory, photo-protective and pro-vitamin A properties [43, 44]. Studies have shown that consuming lutein reduces the risk of age-related macular degeneration, cancers, cardiovascular disease and cataract [43, 45]. Lutein also has antioxidant and photo-protective properties [41, 45, 46]. Astaxanthin exhibits health-promoting effects such as anti-cancers, anti-dermatitis, anti-diabetic, antihypertensive, anti-inflammatory, anti-obesity, antioxidant, cardioprotective, gastroprotective, hepato-protective, hypolipidaemic, immune-modulatory, neuroprotective, nephroprotective and ocular-protective activities [43, 45, 47, 48]. Antheraxanthin is an antioxidant [49]; while violaxanthin and neoxanthin has antioxidant and anti-proliferative activities [45 - 47, 49].

Eleven known saponins were detected in the leaves and stems (**Table 6**), mainly made up of sapogenin (leaves: 62.99%; stems: 64.56%), saponine (leaves: 28. 91%; stems: 24.54%), neochlorogenin (leaves: 8.10%; stems: 10.90%). Twelve known glycosides were detected (**Table 6**), consisting mainly of artemetin (leaves: 65.77%; stems: 67.56%), digitoxin (leaves: 27.65%; stems: 24.81), digoxin (leaves: 5.87%; stems: 6.77%) and cucurbitacin (leaves: 0.68%; stems: 0.55%).

This study showed the artemetin content of *C. aconitifolius* leaves to be comparable to *Artemisia annua* [50]. Artemetin possesses anti-inflammatory, antioxidant, antihypertensive, cardio-protective, immune-modulatory, cell cycle and lipoxygenase inhibitory properties [51, 52, 53, 54]. Digitoxin and digoxin are used in managing atrial fibrillation, congestive cardiac insufficiency, congestive heart failure and cardiac arrhythmias [55, 56]. These promising anticancer agents inhibit Na^+/K^+-ATPase and increase intracellular sodium ions [55, 56]. Cucurbitacins are adaptogenic, anti-atherosclerotic, anti-diabetic, anti-inflammatory, antimicrobial, antioxidant, antitumor, hepatoprotective and immune-modulatory agents [57].

In the leaves and stems of *C. aconitifolius* (**Table 7**), seven known phytosterols were detected, including sitosterol (leaves: 63.64%; stems: 71.28%), stigmasterol (leaves: 13.60%; stems: 10.92%), campesterol (leaves: 12.13%; stems: 6.36%) and 5-avenasterol (leaves: 10.61%; stems: 11.42%). The leaves and stems had lower phytosterol than cabbage and lettuce [21, 58]. They had lower sitosterol than cabbage, but higher contents than lettuce and *T. procumbens* [19, 58]. Their stigmasterol contents were lower than cabbage, lettuce and *T. procumbens* [19, 58]. They had lower campesterol than cabbage, but higher contents than lettuce [58]. They also had higher avenasterol than lettuce [58].

Beta-sitosterol possesses analgesic/anti-nociceptive, angiogenic, anthelmintic, anti-atherosclerosis, anti-arthritic, anticancer, anti-diabetic, anti-hyperlipidaemic, anti-inflammatory, antimicrobial, antioxidant, antipyretic and immunomodulatory activities [8, 59, 60]. According Ikewuchi *et al*. [8] and Saeidnia *et al*. [61], stigmasterol has analgesic, anticonvulsant, anti-hypercholesterolemic, anti-inflammatory, anti-osteoarthritic,

antioxidant, antitumor, hypoglycaemic and memory enhancing activities. Campesterol exhibits anticancer, anti-inflammatory and anti-hypercholesterolemic properties [59, 62]. The antioxidant effect of avenasterols has been reported [63].

Table 4. The amino acids composition of the leaves and stems of *Cnidoscolus aconitifolius*

Compound	Leaves				Stems			
	Retention time (min)	Composition			Retention time (min)	Composition		
		g/100g protein	g/100g sample			g/100g protein	g/100g sample	
			Fresh	Dry			Fresh	Dry
Glycine	8.903	5.698	1.244	1.341	8.652	5.078	1.127	1.240
Alanine	10.646	5.936	1.070	1.153	10.180	4.365	0.969	1.066
Serine	12.098	4.537	0.986	1.062	12.098	4.022	0.893	0.982
Proline	13.740	5.589	1.226	1.321	13.740	5.002	1.110	1.222
Valine*	14.697	5.922	1.251	1.348	14.697	5.107	1.134	1.247
Threonine*	16.040	4.700	1.069	1.152	16.040	4.364	0.969	1.066
Isoleucine*	18.057	4.505	1.182	1.274	16.602	4.826	1.071	1.179
Leucine*	19.027	6.095	1.204	1.297	18.057	4.912	1.091	1.200
Aspartate	19.522	9.100	1.328	1.431	19.522	5.419	1.203	1.324
Lysine*	20.594	6.618	0.835	0.900	21.099	3.408	0.757	0.832
Methionine*	21.674	1.560	0.246	0.265	21.823	1.005	0.223	0.245
Glutamate	22.605	14.601	4.267	4.598	22.605	17.416	3.866	4.253
Phenylalanine*	23.232	5.420	1.193	1.285	23.232	4.868	1.081	1.189
Histidine*	23.968	4.804	0.946	1.019	23.968	3.861	0.857	0.943
Arginine	25.109	5.370	1.985	2.139	24.878	8.100	1.798	1.978
Tyrosine*	25.757	1.651	0.543	0.585	25.616	2.214	0.492	0.541
Tryptophan*	26.250	0.792	0.288	0.310	26.250	1.175	0.261	0.287
Cysteine*	27.114	0.467	0.273	0.295	26.830	1.116	0.248	0.273
Total amino acid content		93.369	21.133	22.773		86.261	19.149	21.067
Total essential amino acids		42.536	9.030	9.730		36.856	8.182	9.001
Total nonessential amino acids		50.833	12.104	13.043		49.403	10.967	12.065
Total sulphur containing amino acids		2.027	0.520	0.560		2.121	0.471	0.518
Total aromatic amino acids		7.864	2.023	2.180		8.258	1.833	2.017

*Essential amino acids

Table 5. Digestible indispensable amino acid (IAA) reference ratios of proteins from the leaves and stems of *Cnidoscolus aconitifolius*

Amino acids	Amino acid composition from present study (mg/g protein)		Digestible Indispensable Amino Acid (IAA) reference ratio					
			Comparison to Infant (birth to 6 months) requirement protein pattern		Comparison to Child (6 months to 3 year) requirement protein pattern		Comparison to older child, adolescent, adult requirement protein pattern	
	Leaves	Stems	Leaves	Stems	Leaves	Stems	Leaves	Stems
Histidine	48.045	38.609	2.288	1.839	2.402	1.931	3.003	2.413
Isoleucine	45.055	48.258	0.819	0.877	1.408	1.508	1.502	1.609
Leucine	60.954	49.122	0.635	0.512	0.924	0.744	0.999	0.805
Lysine	66.182	34.075	0.959	0.494	1.161	0.598	1.379	0.710
Methionine + cysteine	20.270	21.208	0.614	0.643	0.751	0.786	0.881	0.922
Phenylalanine + tyrosine	70.713	70.828	0.752	0.754	1.360	1.362	1.725	1.728
Threonine	46.998	43.642	1.068	0.992	1.516	1.408	1.880	1.746
Tryptophan	7.921	11.753	0.466	0.691	0.932	1.383	1.200	1.781
Valine	59.222	51.067	1.077	0.929	1.377	1.188	1.481	1.277

Table 6. Composition of carotenoids, saponins and glycosides isolated and detected in the leaves and stems of *Cnidoscolus aconitifolius*

Compound	Leaves			Stems		
	Retention time (min)	Composition (mg/kg)		Retention time (min)	Composition (mg/kg)	
		Fresh	Dry		Fresh	Dry
Carotenoids						
Malvidin	19.164	0.0006215	0.0006697	19.166	0.0000683	0.0000751
Beta-cryptoxanthin	20.532	0.0992480	0.1069483	20.535	0.0109350	0.0120297
Lycopene	21.498	0.0000266	0.0000287	21.501	0.0000305	0.0000336
Carotene	22.688	201.0858000	216.6872845	22.688	236.6788000	260.3727173
Lutein	23.228	97.9037000	105.4996767	23.23	112.3721000	123.6216722
Xanthophyll	24.031	34.5080000	37.1853448	24.033	40.5110000	44.5665567
Antheraxanthin	24.876	21.4285000	23.0910560	24.882	39.3176000	43.2536854
Astaxanthin	25.610	6.6838500	7.2024246	25.615	8.3725000	9.2106711
Violaxanthin	26.354	26.7323000	28.8063578	26.355	33.9565000	37.3558856
Neoxanthin	27.164	47.6049000	51.2983836	27.219	70.3723000	77.4172717
Total carotenoids content		541.6908000	469.8781747		436.0470000	595.8105982
Saponins						
Hispigenin	17.547	0.0019419	0.0020926	17.663	0.0007830	0.0008614
Solagenin	18.836	0.0053929	0.0058113	18.759	0.0016800	0.0018482
Diosgenin	19.516	0.0007216	0.0007775	19.514	0.0004930	0.0005424
Tigogenin	19.962	0.0018753	0.0020208	19.954	0.0012800	0.0014081
Neochlorogenin	20.471	26.2613000	28.2988147	20.469	11.9754000	13.1742574
Hecogenin	21.820	0.0004445	0.0004790	21.815	0.0002420	0.0002662
Sapogenin	22.599	204.2939000	220.1442888	22.595	70.9351000	78.0364136
Tribuloin	23.231	0.0043084	0.0046427	23.226	0.0015270	0.0016799
Yanogenin	23.967	0.0043724	0.0047116	23.963	0.0018650	0.0020517
Conyzorgin	24.790	0.0067344	0.0072569	24.786	0.0000432	0.0000475
Saponine	26.355	93.7481000	101.0216595	26.282	26.9639000	29.6632563
Total saponins content		324.3225000	349.4925554		109.8823000	120.8826328
Glycosides						
Arbutin	17.772	0.0147251	0.0158676	17.774	0.0189130	0.0208064
Linamarin	18.054	0.0000005	0.0000006	18.056	0.0000007	0.0000008
Salicin	18.842	0.0015738	0.0016959	18.844	0.0032170	0.0035391
Artemetin	19.103	114.089400	122.9411638	19.105	65.2432000	71.7746975
Amygdalin	19.520	0.0018822	0.0020283	19.522	0.2378190	0.2616271
Ouabain	20.469	0.0214212	0.0230832	20.471	0.0271170	0.0298317
Dhurrin	21.327	0.0011040	0.0011897	21.103	0.0010690	0.0011760
Prunasin	21.437	0.0007409	0.0007984	21.439	0.0009370	0.0010308
Cucurbitacin	21.821	1.1756200	1.2668319	21.909	0.5301180	0.5831881
Digitoxin	22.066	47.9701000	51.6919181	22.068	23.9585000	26.3569857
Digoxin	22.602	10.1820000	10.9719828	22.604	6.5343700	7.1885259
Lotaustralin	23.966	0.00776349	0.0083653	23.968	0.0094160	0.0103586
Total glycosides content		173.4663000	186.9249260		96.5647000	106.2317675

Table 7. Composition of alkaloids isolated and detected in leaves and stems of *Cnidoscolus aconitifolius*

Compound	Leaves			Stems		
	Retention time (min)	Composition (µg/kg)		Retention time (min)	Composition (µg/kg)	
		Fresh	Dry		Fresh	Dry
Trigonelline	7.528	0.0071	0.0077	7.533	0.0059	0.0065
Augustifoline	7.923	0.9063	0.9766	7.925	0.5180	0.5699
Sparteine	8.900	0.0450	0.0484	8.908	0.0166	0.0183
Ellipcine	9.321	0.0255	0.0275	9.320	0.0114	0.0125
Dioscorine	9.925	0.0116	0.0125	9.742	0.0163	0.0179
Serotonin	10.323	2.8847	3.1086	10.353	1.2290	1.3520
Lupanine	10.846	13.6926	14.7550	10.852	5.6810	6.2497
13-Alphahydrorhombifoline	11.054	0.0160	0.0173	11.044	0.0065	0.0071
Dihydrodioscorine	11.352	0.0117	0.0126	11.352	0.0049	0.0054
Zeatin	12.096	0.0331	0.0356	12.094	0.0149	0.0164
9-Octadecenamide	12.821	0.0129	0.0139	12.936	0.0113	0.0124
Vicine	13.419	0.0258	0.0278	13.417	0.0106	0.0117
Dihydro-oxo-demethoxyhaemanthamine	14.151	0.0542	0.0584	14.150	0.0184	0.0202
Augustamine	14.919	0.2208	0.2379	14.916	0.1670	0.1837
Oxoassoanine	15.395	0.0294	0.0317	15.394	0.0119	0.0131
Cinchonidine	16.245	0.0381	0.0411	16.244	0.0154	0.0169
Cinchonine	16.377	0.0329	0.0354	16.366	0.0120	0.0132
Crinane-3alpha-ol	16.452	93.7177	100.9889	16.486	37.2980	41.0319
Buphanidrine	16.672	48.8602	52.6511	16.667	19.6640	21.6326
Protopine	17.093	0.0963	0.1038	17.089	0.0368	0.0405
Alpha allocryptopine	17.362	0.0402	0.0433	17.357	0.0162	0.0178
Indicine-N-oxide	17.548	0.0542	0.0584	17.544	0.0188	0.0209
Tetrahydrocolumbamine	18.054	0.0748	0.0806	18.048	0.0312	0.0343
Coptisine	18.354	0.1208	0.1302	18.349	0.0496	0.0546
Powelline	18.592	42.0523	45.3150	18.585	16.9490	18.6458
Undulatine	18.765	16.4678	17.7455	18.76	7.0410	7.7459
Palmatine	19.400	0.0757	0.0815	19.393	0.0328	0.0361
Ambelline	19.689	88.1140	94.9504	19.675	43.8040	48.1892
Berberine	19.957	0.0053	0.0057	19.955	0.0022	0.0024
6-Hydroxybuphanidrine	20.469	0.0528	0.0569	20.467	0.0237	0.0261
Acronycine	21.102	0.0447	0.0482	21.123	0.0231	0.0254
Monocrotaline	21.328	0.0585	0.0630	21.322	0.0269	0.0296
6-Hydroxypowelline	21.821	0.1139	0.1227	21.816	0.0532	0.0585
Nitidine	22.359	0.0316	0.0341	22.356	0.0153	0.0168
Tetrahydrocoptisine	22.996	0.0283	0.0305	22.991	0.0130	0.0143
Crinamidine	23.967	0.2883	0.3107	23.963	0.1510	0.1661
Echitamidine	26.828	0.0057	0.0061	26.732	0.0037	0.0041
Akuammidine	26.949	984.0220	1060.3685	26.824	489.3980	538.3916
Voacangine	27.06	0.0201	0.0217	27.058	0.0178	0.0196
Mitraphylin	27.425	0.0382	0.0411	27.635	0.0063	0.0070
Echitamine	28.631	0.0016	0.0017	28.629	0.0009	0.0010
Colchicine	28.915	0.0119	0.0128	28.917	0.0082	0.0090
Emetine	29.555	0.0036	0.0039	29.571	0.0024	0.0027
Tetrandrine	29.743	0.0050	0.0054	29.748	0.0039	0.0043
Total alkaloids content		1292.4600	1392.7295		622.4420	684.7548

Table 8. Composition of phytosterols, terpenoids and allicins isolated and detected in the leaves and stems of *Cnidoscolus aconitifolius*

Compound	Leaves			Stems		
	Retention time (min)	Composition (mg/kg)		Retention time (min)	Composition (mg/kg)	
		Fresh	dry		Fresh	Dry
Phytosterols						
Cholesterol	19.600	0.00289	0.00311	19.395	0.00098	0.00108
Cholestanol	20.605	0.00011	0.00012	20.460	0.00005	0.00006
Ergosterol	21.509	0.01836	0.01978	21.394	0.01829	0.02012
Campesterol	22.375	10.86360	11.70647	22.311	4.55842	5.01476
Stigmasterol	23.208	12.18540	13.13082	23.062	7.83465	8.61898
5-Avenasterol	24.005	9.50895	10.24671	23.852	8.18951	9.00936
Sitosterol	25.031	57.01620	61.43987	25.261	51.12140	56.23916
Total phytosterol content		89.59560	96.54688		71.72330	78.90352
Terpenoids						
Taraxerol	19.399	0.01286	0.01386	19.401	0.01402	0.01542
Alpha-amyrin	21.131	2.21037	2.38186	21.102	1.27591	1.40364
Beta-amyrin	21.820	3.65475	3.93831	21.822	4.58234	5.04108
Lupeol	23.229	1.11087	1.19706	23.234	1.02418	1.12671
Bauerenol acetate	24.728	0.01502	0.01619	24.792	0.01304	0.01434
Total terpenoids content		7.00388	7.54728		6.90949	7.60120
Allicins						
Diallyl thiosulphinate	16.448	0.00135	0.00145	16.455	0.01573	0.01731
Methylallyl thiosulphinate	17.699	0.07302	0.07868	17.698	0.11177	0.12295
Allylmethyl thiosulphinate	18.773	0.00670	0.00722	18.744	0.00136	0.00150
Total allicins content		0.09319	0.08736		0.12886	0.14176

Forty four known alkaloids were detected in the leaves and stems (**Table 7**), consisting mainly of akuammidine (leaves: 76.14%; stems: 78.63%), crinane-3alpha-ol (leaves: 7.25%; stems: 5.99%), ambelline (leaves: 6.82%; stems: 7.04%), buphanidrine (leaves: 3.78%; stems: 3.16%), powelline (leaves: 3.25%; stems: 2.72%), undulatine (leaves: 1.27%; stems: 1.13%), lupanine (leaves: 1.06%; stems: 0.91%) and serotonin (leaves: 0.22%; stems: 0.20%). The leaves and stems had lower akuammidine and ambelline, and higher powelline, crinane-3α-ol and buphanidrine than *T. procumbens* [19]. Akuammidine has analgesic, antibacterial, anti-depressant, antifungal, anti-inflammatory, antimalarial, hypotensive and skeletal muscle relaxant activities [19, 64]. Buphanidrine is an antibacterial and sedative [65]. Lupanine is an anti-arrhythmic, bacteriostatic, hypotensive, hypoglycaemic, β-glucosidase inhibitory, central nervous system depressant and oxytocic agent [19].

Three known allicins were detected in the leaves and stems (**Table 8**), consisting of methylallyl thiosulphinate (leaves: 90.07%; stems: 86.73%), allylmethyl thiosulphinate (leaves: 8.27%; stems: 1.06%) and diallyl thiosulphinate (leaves: 1.66%; stems: 12.21%). Five known terpenoids were detected in the leaves and stems (**Table 8**), consisting of beta-amyrin (leaves: 52.18%; stems: 66.32%), alpha-amyrin (leaves: 31.56%; stems: 18.47%), lupeol (leaves: 15.86%; stems: 14.82%), bauerenol acetate (leaves: 0.21%; stems: 0.19%) and taraxerol (leaves: 0.18%; stems: 0.20%). Amyrins (α and β) possesses analgesic, anti-colitis, anticonvulsant, anti-depressive, antifungal, anti-hyperglycaemic, anti- inflammatory, antimicrobial, anti-obesity, antioxidant, anti-pancreatitis, antiplatelet, antipruritic, gastroprotective, hepatoprotective and hypolipidaemic properties [66-68]. Lupeol exhibits anti-arthritic, anti-diabetic, anti-inflammatory, antimicrobial, antiprotozoal, antitumor, cardio-protective and hepatoprotective properties [41, 59, 69, 70].

From the foregoing, it can be concluded that the leaves and stems of *C. aconitifolius* are good sources of macro- and micronutrients, which could be exploited in supplementing the nutrient contents of the human diet. It can also be concluded that the leaves and stems contain a wide range of bioactive phytochemicals. The useful roles of these phytoconstituents can be exploited in the human diet, making them real tools for nutritional therapy. This, therefore, underscores the potential of these leaves and stems as functional foods.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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