



Evaluation of Nutritional, Non-nutritional, Elemental Content and Amino Acid Profile of *Azanza garckeana* (Goron Tula)

I. I. Nkafamiya¹, B. P. Ardo², S. A. Osemeahon¹ and Ayodele Akinterinwa^{1*}

¹Department of Chemistry, Modibbo Adama University of Technology, Yola, Adamawa State, Nigeria.

²Department of Biotechnology, Modibbo Adama University of Technology, Yola, Adamawa State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJAST/2016/19811

Editor(s):

- (1) Ming-Chih Shih, Department of Health and Nutrition Science, Chinese Culture University, Taiwan.
(2) Selvakumar Subbian, Laboratory of Mycobacterial Pathogenesis and Immunity, Public Health Research Institute (PHRI) at Rutgers Biomedical and Health Sciences, Newark, USA.
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Reviewers:

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(2) Julius Ingweye, University of Port Harcourt, Nigeria.
(3) Anonymous, Federal University of Goias, Brazil.

Complete Peer review History: <http://sciencedomain.org/review-history/12077>

Original Research Article

Received 29th June 2015
Accepted 24th September 2015
Published 3rd November 2015

ABSTRACT

Azanza garckeana fruits, leaves, stem-bark and roots were both quantitatively and qualitatively analyzed to determine the nutritional, non-nutritional, elemental and amino acid content. The nutritional (proximate) analysis reveals the highest and lowest moisture content in the fruits (6.50%) and stem-bark (0.50%), crude protein in the fruits (12.00%) and stem-bark (4.91%), crude fibre in the stem-bark (45.30%) and fruits (20.75%), lipid content in the leaves (2.56%) and roots (0.68%), and total ash content in roots (8.70%) and fruits (6.7%), respectively, while vitamin A, B₁, B₂, C and E were all found (in the fruits and leaves), and quantitatively more in the fruits. *A. garckeana* contains alkaloids, tannins, flavonoids, steroids, cardiac glycosides, terpenes, phenols, volatile oils, resins and saponins. However, there is a significant variation in the presence of these compounds in the different parts of this plant. The amount of these compounds were also found to be safe (i.e. below established toxic levels), and of medicinal value. The mineral elements present

*Corresponding author: E-mail: ayoterinwa@yahoo.com;

are also below toxic levels, and may contribute to the dietary requirements of these elements. The amino acid profile reveals 17 types; 7 essential, 2 semi-essential and 8 non-essential in the fruits and leaves in varying amounts.

Keywords: *Azanza garckeana*; nutrition; non-nutrition; mineral elements; amino acids.

1. INTRODUCTION

In many tropical countries, rural dwellers traditionally harvest wide range of leafy vegetables, roots, tubers, and fruits from the wild as food supplements, spices, and sometimes for cultural uses. Labeled as famine or hunger food, some wild plants have been recognized to have the potentials to meet household food and income security [1,2]. The indigenous fruits collected from the wild plays a significant role in food and nutrient security of the poor and rural dwellers. Some wild fruits have been identified to have better nutritional value than cultivated fruits [3,4]. As a result, in recent years, a growing interest has emerged to evaluate various wild edible plants for their nutritional features [5-8]. Like in many African countries, Nigeria's indigenous fruit trees, although undomesticated, play many important roles especially to the people living in rural areas. They are important traditional source of nuts, fruits, spices, leafy vegetables, edible oil and beverages [9]. Like the cultivated vegetables, wild indigenous fruit trees provide vitamins and minerals essential for the proper maintenance of human health [10]. According to FAO [11] and Maghembe et al. [12] the nutritional value of indigenous fruit bearing tree species indicates that many are rich in sugars, essential vitamins and minerals, while others are high in vegetable oil and protein contents. In addition to fruit production and cash, the extensive list of benefits includes firewood, fodder, building material, shade and medicine especially to rural communities. Edible wild leaves and fruits are consumed frequently in Northern Nigeria especially in rural communities where a variety of edible leaves and fruits abound. Some of these are cultivated while others grow in the wild. Several of these wild species bear fruits/leaves during the dry season when cultivated fruits/leaves are scarce [13]. Wild fruits offer a cheap means of providing adequate supplies of mineral, fat, protein and carbohydrate to people living within the tropics [3]. In North eastern part of Nigeria (Gombe State) where common fruits and leaves like bananas and bitter leaves are in short supply, it is possible for wild fruits and leaves of *A. garckeana* (Goron Tula) to provide the vitamin

and mineral requirement of the local populace (Tula people). Affordability as a factor is responsible for the high incidence of malnutrition in low income families that traditionally have a large family size in the study area [13]. Most affected are children of preschool age group with most cases of morbidity related to inadequate intake of food containing essential nutrients [6,13]. Though *A. garckeana* may provide the necessary nutrients needed for body, the availability of these nutrients after ingestion depends on the non-nutritional factors present in the fruits and leaves of the plant. The non-nutrients tend to bind to mineral elements there by forming indigestible complex. Oxalate for instance binds to calcium to form complexes (calcium oxalate crystal). These crystals formed prevent the absorption and utilization of calcium. The calcium crystals may also precipitate around the renal tubules thereby causing renal stones [6,13].

Azanza garckeana is a semi-deciduous tree/shrub with a round medium crooked stem [14,15]. The tree can grow to a height of 3-15 meters depending on the environment in which it is grows [15-19]. The twigs are hairy when young but become smooth with age and branches have wooly hairs [20]. The leaves are distinctively round with 8 to 12 cm long stalks. The leaves have 3 to 5 lobes, which are covered in brown star-shaped hairs, and have longitudinal fissures in the midrib [15,18,20]. The flowers have many stamens and 5 petals, which are yellow or purplish in colour with dark purple or dark red centre [15]. The flowers are bisexual with all floral parts in fives [18]. It flowers in wet season and fruits in dry season (April-August) [18]. The fruits are hairy, spherical, hard and about 2.5 - 4 cm long in diameter, internally divided into 4 to 5 longitudinal sections. They are yellowish to brownish green when mature [15,20]. *A. garckeana* is widely distributed in East and Southern Africa countries like Botswana, Kenya, Malawi, Mozambique, Namibia, South Africa, Tanzania, Zambia and Zimbabwe [15]. ICRAF [20], Mbuya et al. [21] and Mulofwa et al. [19], reported the species growing from Sudan to South Africa. In addition, it is also found in Gombe state, Nigeria, West Africa. The species

grow naturally in all types of woodlands from sea level to about 1700 m above sea level [15,17,19,21]. It grows in semi-arid areas receiving lowest annual rainfall of 250 mm and highest rainfall of 1270 mm [18]. Over the range in its entirety, the species grows in a variety of soils and its found on or near termite mounds and deserted village fields [17,18,19,21]. In Botswana *Azanza garckeana* grows in open woodland in north-eastern part of the country. In Nigeria it grows in savanna areas in Tula District, Kaltungo Local Government Area, Gombe state. The fruits of *A. garckeana* when slightly green or when ripe, are eaten by the people of Tula community in Gombe State, Nigeria. Some people dry it and further process it for consumption. The fruits can also be soaked in small amount of water to make jelly [22]. They can also be boiled and used as relish or made into porridge [14]. Leaves are used for making relish and also cooked as vegetables. The leaves can also be used as green manure to improve land productivity [23]. Traditionally, the roots and steam/bark are used to treat gonorrhoea.



Fig. 1. Typical *A. garckeana* plant

Due to the importance of these nutrients as a basic condition for good human health, we therefore found it necessary to evaluate the nutrient contents of *A. garckeana* fruits and other plant parts. This work would provide necessary information on the fruits, plant parts and also provide the basis for their wider utilization.

2. MATERIALS AND METHODS

2.1 Materials

H₂SO₄, NaOH, HCl, HNO₃, diethyl ether, K₂SO₄, CuSO₄, Na₂CO₃, Fehling's solutions A and B, methyl red indicator, Methylene blue indicator,

standard glucose, ferric chloride, Whatman filter paper, Mayer's reagent, and ethanol used in this research work are analytical grade and products from the British Drug House, (BDH). All materials were used without further purification.

2.2 Collection and Treatment of Samples

A. garckeana (Goron Tula) fruits, leaves, roots and stem-bark (each about 3 kg) were randomly collected from different stands of the plant growing wild in Tula Town in Kaltungo Local Government Area Gombe State in August, 2014. The plant parts (i.e. fruits for 4 weeks, leaves for 2 weeks, stem-bark for 4 weeks, and roots for 4 weeks) were air dried in the laboratory at room temperature. They were then grounded into fine powder using pestle and motor (Stainless steel) and stored in screw capped containers.

2.3 Proximate Analysis

These were carried out by adopting the methods as described in Nkafamiya et al. [24] as follows:

2.3.1 Determination of moisture

Five grams (5.0 g) of each powdered sample (fruits, leaves, stem/bark and roots) were weighed into previously weighed crucibles and dried at 95-100°C for two hours. The samples was removed, cooled in desiccators, weighed and returned into the oven again for an hour. The samples were then brought out and cooled in desiccators before weighing. This was done until a constant weight was obtained with three consecutive weighing. The percentage moisture was calculated thus:

$$\% \text{ moisture} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where

W₁ = weight of empty crucible

W₂ = weight of crucible + sample before drying

W₃ = weight of crucible and sample after drying

2.3.2 Determination of ash

Two grams (2.0 g) of finely ground dry samples obtained after moisture determination was weighed into porcelain crucibles. The samples were charred on a heating mantle inside a fume cupboard to get rid of the smokes. The samples were then being transferred into a muffle furnace and gradually heated to a temperature of 450°C for 3 hours (which was the time a clear grey ash

was obtained). The samples was then cooled in the desiccators and weighed. The percentage ash was calculated thus:

$$\%ash = \frac{\text{weight of ash}}{\text{weight of sample}} \times 100$$

2.3.3 Determination of crude fiber

Two grams (2.0 g) of dried sample powder was boiled in 30 ml of 0.15M H₂SO₄ for 15 minutes, 40 ml of 1.5 M NaOH was added and the boiling continued for 15 minutes. The mixture was filtered after cooling and washed several times with distilled water. The washed residue was placed in a beaker and shaken with 30 ml of a 0.3 M HCl, then filtered. The residue was washed with distilled water and dried in an oven at 105°C for 30 minutes, then weighed and transferred into a muffle furnace for ashing at 100°C for 2 hours. The ash were removed, cooled in desiccators and weighed. The percentage crude fiber was calculated thus:

$$\% \text{ crude fiber} = \frac{WA - WB}{\text{weight of sample}} \times 100$$

Where: WA = weight of residue before ashing
WB = weight after ashing

2.3.4 Determination of lipids

Two grams (2.0 g) of the sample were weighed into 250 ml conical flask, 50 ml of diethyl ether were added, shaken and allowed to stand overnight. The mixture was then filtered over a gravity filtration set and washed down with the same solvent. The filtrate was placed in a water bath to evaporate the ether then dried in an oven at 105°C for 1 hour. The conical flasks with extracts were then weight. The percentage lipid was calculated thus:

$$\% \text{ lipid} = \frac{\text{weight of oil}}{\text{weight of sample}} \times 100$$

2.3.5 Determination of crude protein

Five grams (5.0 g) of the powdered sample were weighed into 500 ml Kjeldahl flask, 10.0 g of K₂SO₄ and 0.5 g CuSO₄ (as catalyst) was added, then 40 ml of 98% H₂SO₄ were also added and the mixture gently heated until fuming ceased. The mixture was further boiled for 2 hours and then cooled to room temperature. Distilled water (250 ml) was added to the digest. The distillation apparatus was set, 50 ml of 0.1 M HCl were

pipette into the receiving flask and the tip of the delivery tube extended just below the surface of the acid, 60cm³ of 50% (w/v) NaOH solution was added to the Kjeldahl flask and the flask was immediately connected to the condenser to complete the set up. The digest solution was heated till it starts boiling. Boiling continued until about 150–200 ml of the digest solution has been collected. The set up was disconnected, distilled, and titrated against 0.1M NaOH using methyl red indicator. The titre value was recorded, and this was used in the calculations from which the percentage nitrogen was obtained thus:

$$\text{Nitro}\%gen = \left(\frac{N_a - N_b \times 0.1 \times 14}{\text{weight of sample}} \times 100 \right)$$

%Crude protein= % N × 6.25

Where N_a= moles of acid (Molarity x Volume)

N_b= moles of base

2.3.6 Determination of carbohydrate

The available carbohydrate content in the samples was determined by difference thus:

% carbohydrate = 100 – (% moisture + % ash + % crude protein + % lipid extract + % crude fibre).

2.3.7 Determination of vitamin (A, B₁, B₂, C, and E) content (leaves and fruits)

Vitamin content was determined according to the method adopted by Nkafamiya et al. [6] as follows: β-carotene in the samples was chromatographically determined, and the formula; 0.6 μg of β-carothene = 0.3 μg of pure vitamin A. other vitamins were spectrophotometrically determined, and the mean values from triple determinations were recorded.

2.3.8 Analysis of minerals and amino acids (leaves and fruits)

Two gram (2 g) of each sample was weighed into separate beakers and treated with 20 ml of HNO₃. It was then digested on an electric hot plate at 70°- 90° C for 60 minutes. Blank were then prepared similarly by digesting 20 ml of HNO₃ acid in an empty beaker. It was then cooled and the content was filtered through Whatman No. 42 filter paper into a volumetric flask and made up to 100 ml with deionized water. The digests was then analyzed for the elemental contents using an atomic absorption spectrophotometer (AAS), Buck 210 VGP. Amino

acid profile analysis was carried out according to standard methods as adopted in Nkafamiya et al. [24].

2.3.9 Determination of non-nutrients

These were carried out by adopting the methods described in Idris et al. [25] and Barminas et al. [26]. As follows:

2.3.9.1 Test for alkaloids

To 3 ml of the extract in a test tube, 1 ml of 1% HCl was added. The mixture was heated for 20 minutes cooled and filtered. About 2 drops of Mayer's reagent was added to 1 ml of the filtrate. A creamy precipitate indicates the presence of alkaloids.

2.3.9.2 Test for saponins

Frothing test: 2 ml of the extract was vigorously shaken in a test tube for 2 minutes and observe for frothing. Emulsion test: 5 drops of olive oil was added to 3 ml of the extract in the test tube and vigorously shaken, the absence of frothing and stable emulsion indicates the absence of saponins.

2.3.9.3 Test for tannins

About 0.5 g of the dried powder sample was boiled in 20 ml of water in a test tube and then filter. A few drops of 0.1% ferric chloride was added and observe for brownish green or a blue-black coloration.

2.3.9.4 Test for flavonoids

To 3 ml of the extract, 1 ml of 10% NaOH was added. The absence of flavonoids shows yellow coloration.

2.3.9.5 Test for steroids

To 0.5 g extract of the sample, 2 ml of acetic anhydride and 2 ml H₂SO₄ was added. The color change from violet to blue or green indicates the presence of steroids.

2.3.9.6 Test for terpenoids

To 5 ml of the extract, 2 ml of chloroform was added and mixed, 3 ml of concentrated H₂SO₄ was carefully added to form layers and a reddish brown coloration of the interface was formed, indicating the presence of terpenoids.

2.3.9.7 Test for cardiac glycosides (CGs)

To 0.5 g of the extract dissolved in 2 ml of chloroform. Sulphuric acid was carefully added to form layers. A redish-brown colour at the interface indicates the presence of a steroidal ring.

2.3.9.8 Test for volatile oils

To 10 ml of extract was added 50 ml of 90% ethanol and few drops of ferric chloride solution. A blue coloration was recorded as the presence of volatile oils.

2.3.9.9 Test for resins

About 2 ml aliquot of extract and equal volume of acetic anhydride solution were added followed by drops of conc. H₂SO₄. A violet coloration was recorded as the presence of resins.

2.3.9.10 Test for Phenols

To 10 ml of extract was added 10 ml of ferric chloride solution. A deep blue coloration was recorded as the presence of Phenols.

2.4 Quantitative Analysis of Phytochemicals

The quantitative analyses of the phytochemicals were carried out using standard methods described by Monika et al., [11] and AOAC, [27]. In all the analysis, reagents were of analytical grades and were not subjected to further purification.

3. RESULTS AND DISCUSSION

The results of the proximate analysis of *A. garckeana* fruits, leaves, roots and stem-bark are presented in Table 1. The fruit is the most proteinous part (12.0 %) and also with the highest moisture content (6.50 %), while the stem-bark is the least (4.91%, protein and 0.5 % moisture content). The stem-bark contains the highest carbohydrate content (72.16 %), closely followed by roots (70.81 %), while the least carbohydrate content is obtained from the fruits (28.40 %). Crude fiber content was found to be highest in fruits (45.00 %) followed by leaves (25.00 %), stem-bark (13.75 %) and roots (11.89 %). This considerable amount of crude fiber implies that *A. garckeana* (especially the fruits and leaves) will perform the role of stool softening, with optimum frequency and regularity

of elimination, which is characteristic of fibre-rich diet (Nkafamiya et al.). The highest total ash content was found in the leaves (11.00%), and the least in the fruits (6.70%) (w/w). Comparing this result with the one reported by Nkafamiya et al. [24] for *F. asperifolia* and *F. sycomorus*, the ash contents are comparable, while the other results obtained for *A. garckeana* parts are correspondingly lower. However, values obtained are within the range expected for dry leaf vegetables [24].

The non-nutritional component of the fruits, leaves, stem-bark and roots are presented in Table 2 (qualitative) and Table 3 (quantitative). These are compounds that limit the wide use of many tropical plants due to their ubiquitous occurrence as natural compounds capable of eliciting deleterious effect on man and animals [24,28]. Alkaloids, steroids, cardiac glycosides, terpenes, resins and saponins are present in the plant parts (fruits, leaves, roots and stem-bark). Volatile oils are present in the fruits, leaves and the roots. Flavonoids are present in the fruits and leaves. Phenols and tannins are present only in leaves and fruits respectively. This result (especially the fruits and leaves) is comparable with those reported for some medicinal plants by Kubmarawa et al. [29] and Idris et al. [25].

The quantitative estimation of the non-nutritional factors (Table 3) indicated that alkaloid has the highest value in fruits (18.40%) and lowest in roots (6.80%). Leaves has the highest value of flavonoids (26.50%) followed by the fruits (24.40%). Flavonoids were not detected in the root and stem-bark. Saponins are not also detected in the fruits (concentration is below the detection limit of the procedure used), but has the highest value in leaves (30.00%) followed by the stem-bark (34.50%). Tannins were not detected in the leaves (concentration is below the detection limit of the procedure used), roots and stem-bark, but amount to 15.05% in the fruits. Phenols quantitatively amount to 29.00% of the plant leaves where it was only found. Although very medicinal [25], non-nutritional compounds above some threshold intake can bind to mineral elements there by forming indigestible complex. Oxalate for instance tends to render calcium unavailable by binding to the calcium ion to form complexes (calcium oxalate crystals). These oxalate crystals formed prevents the absorption and utilization of the calcium. The calcium crystals may also precipitate around the

renal tubules thereby causing renal stones [24]. The non-nutritional compounds present in the plant parts are generally below the established toxic level [13,24]. This thereby potentially recommends the plant parts (especially the fruits and leaves) for medicinal uses.

Table 4 presents some mineral composition of the fruits and other plant parts. The levels of mineral elements in fruits are higher than those found in the fruits of *C. congoensis*, *N. latifolia* and *C. panados* [13,30]. Iron and zinc are among the essential elements for humans, and their amount (especially in the fruits and leaves) of *A. garckeana* are promising since the adult daily requirements are 15 and 18 mg, respectively. The iron level is also higher than some of the cultivated fruits like orange (0.2 mg/100 g) and mango (0.4 mg/100 g) [13]. Compared to the other parts, the fruits and leaves of *A. Garckeana* generally have appreciable levels of mineral elements. Therefore, consumption of *A. Garckeana* parts, specifically the fruits and leaves, could contribute immensely to the overall daily intake of these elements in amounts that will alleviate symptoms associated with their deficiency such as; hypercholesterolemia, demineralization of bones, microcytic anemia and immunocompetence (in pregnant and menstruating females) resulting from copper and iron deficiency, weakness, cardiac arrhythmia, poor growth, impairment of sexual development and poor wound healing as a result of magnesium and zinc deficiency [13].

The vitamin content of the fruits and leaves of *A. Garckeana* is presented in Table 5. The vitamin content with the exception of vitamin C is higher compared to those found in the fruits of *C. congoensis* and *N. latifolia* [13]. Consumption of the fruits and leaves of *A. garckeana* can serve as vitamin supplements. Presence and amount of vitamin A and E in the fruits and leaves presents them as a potential remedy for symptoms associable with the deficiency of the vitamins e.g. night blindness and peroxidation. The value of vitamin C in both fruits and leaves of *A. garckeana* is low compared to the fruits of *C. congoensis* (410.50±0.32 mg/100 g ripe) and *N. latifolia* (309.00 mg/100 g ripe). Though the value of vitamin C of *A. garckeana* is low compared to those of the fruits of *Congoensis* and *N. latifolia*, the fruits and leaves may prove important in the prevention of scurvy and alleviate symptoms of common cold [13].

Table 1. Proximate composition of *A. garckeana* (%)

Part of plant	Moisture content (w/w)	Crude protein (w/w)	Crude fibre (w/w)	Lipid content (w/w)	Total ash (w/w)	Total carbohydrate (w/w)
Fruits	6.50	12.00	45.30	1.10	6.70	28.40
Leaves	5.50	5.60	25.00	0.96	11.00	49.94
Roots	2.70	7.42	11.89	0.68	8.70	70.81
Stem/bark	0.50	4.91	13.75	1.12	7.56	72.16

Table 2. Qualitative screening of non-nutritional constituents of *A. garckeana*

Part of plant used	Alkaloids	Tannins	Flavoniods	Steroids	Cardiac glycosides	Terpenes	Phenols	Volatile oils	Resins	Saponins
Fruits	+	+	+	+	+	+	-	+	+	+
Leaves	+	-	+	+	+	+	+	+	+	+
Roots	+	-	-	+	+	+	-	+	+	+
Steam-bark	+	-	-	+	+	+	-	-	+	+

Key: + present; - Not detected

Table 3. Quantitative screening of non-nutritional constituents

Part of plant used	Alkaloids % (w/w)	Flavoniods % (w/w)	Saponins % (w/w)	Tannins % (w/w)	Phenols % (w/w)
Fruits	18.40	24.40	ND	15.05	ND
Leaves	13.60	26.50	30.00	ND	29.00
Roots	6.80	ND	24.50	ND	ND
Steam bark	12.80	ND	34.50	ND	ND

Key: ND not detected

Table 4. Concentration of mineral elements in plant parts (mg/100 g)

Element	Fruits	Leaves	Roots	Stem/bark
Ca	127±0.04	129±0.45	100±0.04	28.02±0.89
Cd	ND	ND	ND	ND
Co	0.02±0.01	0.04±0.02	0.01±0.25	0.01±0.91
Cr	ND	ND	ND	ND
Cu	0.45±0.33	2.01±0.25	0.01±0.35	0.12±0.97
Fe	12.00±0.43	15.00±0.73	5.05±0.23	6.00±0.36
Mg	96.25±0.67	100.00±0.12	45.05±0.24	40.09±0.45
Mn	0.02±0.23	0.01±0.24	0.01±0.98	0.01±0.24
Ni	ND	ND	ND	ND
P	30±0.87	29.07±0.07	10.09±0.90	9.09±0.67
Pb	ND	ND	ND	ND
Zn	12.02±09	11.06±21	6.09±07	5.06±23

Table 5. Vitamin content (mg/100 g) of *A. garckeana* fruits and leaves

Fruits/leaves	A	B ₁	B ₂	C	E
Fruits	75.00±0.23	1.28±0.97	1.18±0.45	319.09±0.45	3.08±0.55
Leaves	28.75±0.66	1.00±0.67	0.95±0.78	98.02±0.65	2.09±0/77

• Value in µg/100 g

Values are means ± SD of 3 determinations

Table 6 present the amino acid composition of *A. garckeana*. Essential amino acids were present in both the fruits and leaves and are higher than that of *F. asperifolia* and *F. sycomor* [24]. Leucine had the highest value for both the fruits and the leaves, while glycine had the lowest. Result is comparable with the result presented by Nkafamiya and his coworkers [24].

Table 6. Amino acid composition of the fruits and leaves of *A. garckeana* (g/100 g)

Amino acid	Fruits	Leaves
Alanine	3.30	4.00
Arginine	7.01	7.69
Aspartic acid	9.67	10.97
Cysteine	3.00	3.66
Glycine	1.00	1.23
Glutamic acid	10.79	11.09
Histidine	3.67	4.00
Isoleucine	4.98	5.00
Leucine	12.01	12.97
Lysine	11.78	12.85
Methionine	2.00	2.78
Phenylalanine	8.00	9.00
Proline	4.00	4.78
Serine	3.97	4.00
Threonine	4.78	4.97
Tyrosine	4.89	4.99
Valine	6.00	6.76

4. CONCLUSION

This study presents the nutritional evaluation of *A. garckeana* which is a wild plant. *A. garckeana*

contains appreciable levels of nutrients in its various parts (especially the fruits and leaves). The presence and amount of some phytochemicals and mineral elements analyzed has also indicated that the consumption of the plant (especially the fruits and leaves) can be of medicinal and dietary values respectively. The 2 semi-essential (Arginine and Histidine), and 7 out of the 8 essential amino acids were found in appreciable amounts in the fruits and leaves. This consistently indicates the high proteinous content of the plant parts. The study therefore further recommends the consumption of *A. garckeana* (especially the fruits and leaves), while other parts may serve as raw materials for nutrients and medicinal substance extraction.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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