

Assessment of Nutrients, Antinutrients and In Vitro Antioxidants Studies of Whole Leaf, and Leaf Extract of *Azanza garckeana*

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Abstract

Plants-derived functional foods have been a significant source of nutrients and medicinal compounds. The purpose of this study was to evaluate the nutritional values, antinutrients compositions, and *in vitro* antioxidants activities of methanolic leaf extract of *Azanza garckeana*. The analysis were carried out using standard analytical methods. Results of the proximate compositions were carbohydrates (69.18 %), protein (10.51 %), fats (7.33 %), fibres (5.27 %), moisture (4.38 %), and ash (3.57 %). The total energy value was 384.7 kcal/g. The vitamins analysis showed the presence of vitamin A (1.876 ± 0.060 mg/100g), C (0.050 ± 0.012 mg/100g), E (0.606 ± 0.009 mg/100g), D (0.197 ± 0.076 mg/100g), K (0.164 ± 0.012 mg/100g), B1 (0.739 ± 0.019 mg/100g), B2 (0.37 ± 0.005 mg/100g), B3 (0.155 ± 0.033 mg/100g), B6 (0.446 ± 0.022 mg/100g) and vitamin B12 (0.157 ± 0.0026 mg/100g). The mineral compositions revealed the presence of sodium (815.287 ± 0.004 mg/100g), calcium (731.515 ± 0.003 mg/100g) and copper (1.048 ± 0.008 mg/100g). Antinutrients composition revealed phytate (1.567 ± 0.005 mg/100g), trypsin inhibitor (0.383 ± 0.006 Tiu/mg), oxalate (0.867 ± 0.003 mg/100g), hemagglutinin (0.741 ± 0.004 Hui/mg), and saponins (0.546 ± 0.004 mg/100g). The antioxidants activity of the methanolic crude extract of *A. garckeana* leaf showed that the total antioxidants capacity (TAC) of the plant extract increased in concentration when compared to the standard antioxidant (ascorbic acid). While the methanolic leaf extract exhibited higher FRAP activity across all the concentrations when compared with the standard antioxidant - butylated hydroxytoluene (BHT). The results of the present study showed that the evaluated leaves of *A. garckeana* plant is rich in nutritional values, have potent antioxidants and essential nutrients with therapeutics properties.

Introduction

Plant-derived functional foods have gradually become an area of increasing research interest because of their health benefits to the human body [1]. Functional foods contain physiologically active constituents that provide an additional health benefit beyond basic nutrition. This may include some plants products containing proven and well documented physiologically active compounds that may improve human health and reduce the risk of chronic diseases [2]. Plants have been considered a significant source of medicinal nutrients and compounds [3]. In Africa, research has shown varieties of fruits bearing plants exists and play a significant role in the diet of the populace. Fruits from plants serve as food and source of nutrients, because they provide some of the minerals that are essential for body building and regulate some body functions such as metabolism. Also, they provide energy that is required for the body activities [4]. Most foods from the wild play an important role in supplying nutrition during periods of food scarcity [5]. Foods are good sources of vitamins, minerals and dietary fibers as well as phytochemicals, which are beneficial for the human body as nutritional supplements [6].

The plant *Azanza garckeana* (F. Hoffm). Exell and Hillc., is a tropical fruit plant found in Africa, locally known as Goron Tula, Jakjak and *Thespesia garckeana* among different local names in tropical Africa, belong to the Malvaceae family. *Azanza garckeana* fruit is an important plant for food and herbal medicine in Africa. The tree is found in Botswana, Malawi, Zambia and Zimbabwe in South Africa; it is

also present in Burundi, Kenya and Tanzania in East Africa; it can also be found in Democratic Republic of Congo in Central Africa; Sudan in North Africa; and Nigeria in West Africa where it is abundantly found in Tula, Gombe State, Kankiya, Katsina State and Daggish Kali highlands of Zah district, Adamawa State. Jakjak is an important food and medicinal plant in tropical Africa. In addition, jakjak is a popular multipurpose fruit, particularly used during seasonal food shortages, and is often the only available food source of high nutrients [7–9]. The genus name “*Azanza*” is probably derived from the word “Azania” an old word in Zanzibar Tanzania in East Africa, meaning black and the specie *garckeana* was named after the German botanist Christian August Friedrich Garcke [8]. The fruit however continues to attract attention as it is claimed to increase fertility, female libido and heightening sexual sensation for women with no side effects. This has demand for the fruit throughout the year round from many parts of the country [10]. *A. garckeana* is also a potential source of fertility compounds, possessing both spermatogenic and aphrodisiac activities, which may be utilized in traditional or alternative health-care systems, owing to its fast action in boosting energy for sexual satisfaction [9]. Though *A. garckeana* may provide the necessary nutrients needed for the 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 [11]. Thus, this study was designed to assess the nutritional properties, anti-nutrients values and evaluate health promoting antioxidants activities of *A. garckeana* leaves with the potential for various health benefits, alleviating micronutrients malnutrition and protein energy malnutrition (PEN). The outcome of this findings will aid in developing baseline data on the dietary phytonutrients and nutraceutical prospects of *A. garckeana* leaves, which can further be utilized in the preparation of dietary supplement and functional foods.

Materials and Methods

Materials

Collection and Authentication of Plant Material

Fresh leaves of *A. garckeana* were collected from Tula, Kaltungo Local Government Area of Gombe State, Nigeria in December, 2022. The leaves were identified and authenticated by Mr. Alfred Ozioko, a Botanist at the International Centre for Ethnomedicine and Drug Development (InterCEDD), Nsukka, Enugu State, Nigeria. The specimen was assigned a voucher number: InterCEDD/3459Aza and was later deposited at the Herbarium of InterCEDD for reference purposes.

Methods

Preparation of Plant Material

The fresh leaves of *A. garckeana* were air dried at room temperature and pulverized into a fine powder using a mechanical grinder. The powdered leaves (1,000g) were subjected to extraction by cold maceration method using absolute methanol. The mixture was shaken intermittently at room temperature for 48 hours. Thereafter, the sample was filtered with a muslin cloth and further with Whatman grade 1 qualitative filter papers. The filtrate was concentrated by vacuum evaporation, and the dried extract was stored in a sterile screw-capped bottle and stored in cool place until needed for further analysis. The percentage yield of the methanol extract was determined from the weight of the dried pulverized leaves before maceration and the weight of the crude extract after concentration following the formula below:

$$\text{Percentage Yield of the Crude Extract (\%)} = \frac{\text{Weight of Crude Extract (g)}}{\text{Weight of Dried Pulverized Sample (g)}} \times 100$$

Nutritional and Mineral Analysis of *A. garckeana* Leaves.

Proximate Analysis of *A. garckeana* Leaves

Total carbohydrates, total ash, crude fiber, crude fat, protein and moisture content were determined using standard method as described by [11, 12].

Determination of Energy Content of *A. garckeana* Leaves

The total energy value in the sample was estimated in kilocalories per gram and calculated by multiplying the values obtained for carbohydrate, crude fat and crude protein and adding up the values as describe by [11, 12] using the factors; 4 Kcal/g, 9 kcal/g and 4 kcal/g respectively as shown below:

Total energy value (kcal/g) = (%Crude protein × 4 kcal/g) + (%crude fat × 9 kcal/g) + (%carbohydrate × 4 kcal/g).

The % total kcal. from nutrients in the samples was estimated as follows:

$$\% \text{ kcal from each nutrient} = \frac{\text{kcal from nutrient}}{\text{Total kcal}} \times 100/1$$

$$\% \text{ of total kcal from each nutrient} = \frac{\text{Kcal. from nutrients (carbs + protein + fat)}}{\text{Total kcal}} \times 100/1$$

Determination of Vitamins Content of the Leaves of *A. garckeana*

The vitamins K, D and B12 were determined according to the methods of the Association of Official Analytical Chemists [13]. Vitamin A was determined using the method described by Alexander and Griffiths [14]. Vitamin C was determined using the 2, 6-dichlorophenolindophenol (DPIP) dye method described by Jones and Hughes [15]. Vitamin E was determined using the method described by Pearson [16]. Vitamin B1, B2 and B3 were determined according to the method described by Snell and Snell [17].

Determination of Minerals Content of *A. garckeana* Leaves

Minerals contents (calcium, magnesium, and copper) were determined using the Atomic Absorption Spectrophotometer (AAS) (UNICAM 960 series) as described by AOAC [18]. Iron was determined according to Phenanthroline method as described in AOAC [18]. Zinc was determined according to Pearson method [16]. Sodium and potassium were determined using flame photometric method AOAC [18] by running the sample filtrate in a flame photometer with standard sodium and potassium.

Determination of Antinutrients Compositions of *A. garckeana* Leaves

The antinutrients compositions of the dried leaves of *A. garckeana* was determined as follows: the amount of oxalate was determined using the method described by Sanchez-Alonso and Lachica [19]. Trypsin inhibitor and Phytic acid were determined according to the method described by Sadasivam and Manickam [20]. Saponin content was determined according to the method described by Okwu and Josiah [21].

In vitro Antioxidants Assays of *A. garckeana* Leaves.

The *In vitro* antioxidant activities of *A. garckeana* leaves were assayed according to the method described by Prieto *et al.* [22]; and Alachaher *et al.* [23]. Methanol extracts of *Azanza garckeana* leaves at varying concentrations (0.1 – 0.5 µg/mL) were evaluated for antioxidants activities using Fe³⁺ ion reducing power (FRAP) and total antioxidant capacity (TAC). The extract concentration providing 50% inhibition (IC₅₀) was calculated from the plot of inhibition (%) against extract concentration. Ascorbic acid at the same concentrations was used as the reference antioxidants for total antioxidants capacity (TAC), while butylated hydroxytoluene (BHT) was used as the standard for ferric ion reducing antioxidant power (FRAP).

Analysis of Total Antioxidants Capacity (TAC) of Methanolic Leaves Extract of *A. garckeana*

This was carried out according to the method described by Prieto *et al.* [22]. Different concentrations (0.1 - 0.5 µg /ml) of the *A. garckeana* methanolic leaves extract were taken into test tubes. 3ml of phosphomolybdenum reagent (28 mM sodium phosphate and 4 mM ammonium molybdate in 0.6 M H₂SO₄, then was incubated for 90 min. at 95 °C, after cooling to room temperature, the absorbance was taken at 695 nm against a black. The experiment was performed thrice and results were averaged. Radical scavenging activity was expressed as the inhibition percentage and was calculated using the following formula:

$$\% \text{ Scavenging Activity} = [(A_{695} \text{ Ctrl} - A_{695} \text{ treatment}) / A_{695} \text{ Ctrl}] \times 100.$$

Ferric ion Reducing Antioxidant Power (FRAP) Assay of Methanolic Leaves Extract of *A. garckeana*

The oxidant in the FRAP assay was prepared by mixing TPTZ, acetate buffer, and FeCl₃.H₂O. The conglomerate is referred to as “FRAP reagent”. To measure FRAP value, freshly prepared FRAP reagent

was warmed to 37 °C and a reagent blank reading was taken at λ 593 nm, then the sample and 30 μ L of water are added. Absorbance readings are taken at λ 593 nm. The concentration that causes an increase in the initial FRAP concentration by 50% is defined as IC₅₀.

Procedure for Analysis of Ferric Reducing Antioxidants Power (FRAP) of Methanolic Leaves Extract of *A. garckeana*

This was carried out according to the method described by Alachaher *et al.* [23]. 1 mL of solution containing 0.1, 0.2, 0.3, 0.4 and 0.5 μ g/ml of the methanolic leaves extracts of *A. garckeana* was mixed with sodium phosphate buffer (5.0 mL, 0.2 M, pH 6.6) and potassium ferricyanide (5.0 mL, 1.0%). The mixture was incubated at 50 °C for 20 minutes. Then 5 mL of 10% trichloroacetic acid was added and centrifuged at 1000xg. The upper layer of the solution (5.0 mL) was diluted with 5.0 mL of distilled water and ferric chloride and absorbance is measured at 700 nm. The experiment was performed thrice and results were averaged. Radical scavenging activity was expressed as the inhibition percentage and was calculated using the following formula:

$$\% \text{ Scavenging Activity} = [(A_{700\text{nm}} \text{ Ctrl} - A_{700\text{nm}} \text{ treatment}) / A_{700\text{nm}} \text{ Ctrl}] \times 100.$$

Statistical Analysis

Data collected were subjected to statistical analysis using Microsoft Excel statistical analysis package. All experiments were performed in triplicate (n = 3) and results were expressed as Mean $\pm$ Standard Deviation.

Results and Discussion

Due to the vast levels of macro and micronutrients in wild plants, their role in value addition to deficient foods and health cannot be underestimated [2]. The consumers' demands have changed from energy providing diet to a diet with a balanced nutrient profile along with metabolic, physiological and functional health benefits [24]. *Azanza garckeana* plant has continued to attract interest in its bioactivities, cytotoxicity and antioxidant activity [10]. *A. garckana* leaf was evaluated for its nutritional potentials and antioxidants activities using standard analytical methods. The results of the present research are discussed in the following headings:

Percentage Yield of Methanol Extract of *A. garckeana* Leaves

The extraction of 1000 g of dried crude sample of *A. garckeana* leaves with methanol gave an extract yield of 152.6 g which gave percentage yield of 5.69% of the starting plant material.

Nutritional and Mineral Compositions of *A. garckeana* Leaves

Total Energy Value (Caloric Value), and Percentage (%) Yield of *A. garckeana* Leaves

The total energy value (caloric value) and percentage yield of *A. garckeana* leaves was 384.7 kcal/g (100 %). This implies that the values of food energy found in *A. garckeana* leaves were: 276.7 kcal/g of carbohydrate (72%), 42.0 kcal/g of protein (11%), and 65.9 kcal/g of fat (17%) respectively as shown in **Table 2**.

Proximate Compositions of *A. garckeana* Leaves

Balanced diets are essential for normal growth, health and preservation of the human body. The proximate compositions of the dried leaves of *A. garckeana* showed considerable amount of carbohydrates (69.18 %) with varying concentrations of protein (10.51 %), fats (7.33 %), fibres (5.27 %), moisture (4.38 %), ash (3.57 %) presented in **Table 1**. These values are comparable to the findings of [2] who observed that the fruits of *A. garckeana* have been reported to be high in fibre, total carbohydrates, protein level, ash and low in fat. This study revealed moderate protein contents (10.51%) in the leaves of *A. garckeana* plant (**Table 1**). Similar findings have been reported by Sirajo *et al.* [25], who reported similar crude protein contents (10.01%) in the peel of *A. garckeana*. Protein is an essential component of human diet which is needed for the replacement of tissue and supply of energy. Protein deficiency can cause growth retardation, muscle wasting, oedema, and abnormal swelling of the body [26]. It is important to note, especially for higher risk groups such as older individuals, that adequate intake of both calories and protein will help prevent malnutrition [27]. In this research, the leaves of *A. garckeana* was shown to be considerably rich in carbohydrates which are macronutrients with an exclusively energetic function (4 Kcal/g). They are indispensable for the metabolism of erythrocytes and vital organs such as kidney and brain [28]. This study revealed that the leaves of *A. garckeana* contained 3.57% ash content (**Table 1**). Similarly, 11.00% total ash content has been previously reported for *A. garckeana* leaves [11]. This discrepancy could be attributed to the ecological variations where the wild edible plant is commonly found. The ash content gives a measure of total amount of inorganic compounds like minerals present in a food [29]. Mineral elements are the essential constituents of ash [30]. Fat is one of the major component causing differences in the gross energy of various food substance, it yields over 9 Kcal/g compared to carbohydrates and proteins that yield about 4 kcal/g [12]. Thus, lipids are the macronutrients with higher calorie density. 7.33 % of fats (65.9 kcal/g) was found in the leaves of *A. garckeana*, a considerable amount. Hence, lipids have both a plastic and energetic function in the first half of life, containing essential nutrients such as polyunsaturated fatty acids (LC PUFA) and their conditionally essential longer chain derivatives, in particular arachidonic acid (ARA), and DHA; they also allow the absorption of fat-soluble vitamins. These nutrients contribute to modulate the inflammatory response, regulate gene expression and influence the fluidity of cell membranes of various tissues, including those having a high functional specialization, such as brain and retina [28]. The whole leaf of *A. garckeana* plant have a total energy value (caloric value) of 384.7 kcal/g represented in **Table 2**, which implies that the value of food energy found in the leaves of *A. garckeana* plant is considerably high. Therefore, it can be used to supplement the daily energy intake of the people. However, the energy value is comparatively lower than the food energy obtained for the seeds of *C. congoensis* 4436 ± 1.04 kcal/kg, *B. aegytiaca* 4560 ± 0.07 kcal/kg, *D. Microcarpum* 4448 ± 0.57 kcal/kg, *G. arboea* 4446 ± 0.09 kcal/kg and *N. latifolia* 4337 ± 1.12 kcal/kg as reported by Nkafamiya *et al.* [31]. It was also found that

the leaves of *A. garckeana* plant contains 4.38 % moisture content (**Table 1**) lower than that reported by Sirajo *et al.* [25], who reported that the peel and seed of *A. garckeana* contained 6.66% and 5.00% moistures respectively. The moisture content is a major determinant in the healing, safeguarding and sustenance of food and drug. A high moisture value will promote the activities of spoilage micro-organisms and result in reduced shelf life [30]. The results showed that the leaves sample of *A. garckeana* have a minimal moisture content and considerable amount of ash content which are very important factors required for checking quality for both utilization and storage purposes. As well as its contained food fibers which have been shown to aid the absorption of dietary minerals as well as reduce the absorption of cholesterol and help to maintain blood sugar. Diets rich in fibres helps to prevent constipation, support the health of the digestive tract as well as avert colon cancer [30]. Dietary fibres is not an essential nutrient because it does not have an energy function. However, it regulates intestinal function by influencing the microbiota, increasing the fecal mass and accelerating the intestinal transit time [28]. These may account for the observed potentials of the leaves of *A. garckeana* plant in the treatment and management of diseases such as coronary heart disease, cancer, diabetes, edema, epilepsy, liver problems and sexual transmitted diseases [32, 33].

Table 1: Proximate Compositions of *A. garckean* Leaves

Proximate Compositions (w/w)	Values (%)
Moisture Content	4.382±0.006
Ash Content	3.570±0.003
Fats Content	7.332±0.432
Protein	10.514±0.004
Fibre	5.269±0.052
Carbohydrates	69.181±0.052

Results were expressed as Mean ± SD of three determinations (n = 3).

Table 2: Total Energy Value (Caloric Value), and Percentage (%) Yield of *A. garckeana* Leaves.

Energy Yielding Nutrients	Kcal/g	Kcal (%)
Carbohydrates	276.7	72%
Protein	42.0	11%
Fat	65.9	17%
Total Kilocalories (kcal.)	384.7	
Total Kcal. (%)		100%

Results are expressed as Mean ± SD of three determinations (n = 3).

Vitamins Compositions of *A. garckeana* Leaves

The result of the vitamins composition found in the leaves of *A. garckeana* showed the presence of vitamin A (1.876 ± 0.060 mg/100g), vitamin C (0.050 ± 0.012 mg/100g), vitamin E (0.606 ± 0.009 mg/100g), vitamin D (0.197 ± 0.076 mg/100g), vitamin K (0.164 ± 0.012 mg/100g), vitamin B1 (0.739 ± 0.019 mg/100g), vitamin B2 (0.37 ± 0.005 mg/100g), vitamin B3 (0.155 ± 0.033 mg/100g), vitamin B6 (0.446 ± 0.022 mg/100g) and vitamin B12 (0.157 ± 0.0026 mg/100g) as shown in **Figure 1** & supplementary **Table 3** in varied proportions. A bidirectional relationship exists between nutrition and infection, whereby poor nutritional status predisposes one to infection and where infection is exacerbated by a poor nutritional status. Vitamins and minerals play a role in neutralizing the effects of harmful oxidative agents that have the potential to damage cells [27, 29]. Vitamins are organic compounds (or related set of molecules) which are essential micronutrients that organisms including humans require in minute quantities, for proper functioning of their metabolism. Essential nutrients cannot be made or synthesized in the human body (organism), and therefore must be obtained by the diet [29]. The leaves of *A. garckeana* plant was found to contained both fat and water soluble vitamins in varied concentrations as shown in **Figure 1** & supplementary **Table 3**. The report is in agreement with the findings of Alagbe *et al.* [34], who recorded β -carotene 1.97 ± 0.001 mg/100g, vitamin B1 (Thiamine) 0.74 ± 0.003 mg/100g, vitamin B2 (Riboflavin) 0.42 ± 0.002 mg/100g, vitamin B3 (Niacin) 0.30 ± 0.004 mg/100g, vitamin B6 (Pyridoxine) 0.22 ± 0.005 mg/100g, vitamin B12 (Cyanocobalamin) 0.18 ± 0.007 mg/100g, vitamin D (Calciferol) 0.13 ± 0.001 mg/100g, and vitamin K (Phytonadione) 0.10 ± 0.001 mg/100g for *Danielle Oliveri* Stem Bark respectively. The water-soluble vitamin B and C are involved in various metabolic reactions. Riboflavin (vitamin B2), niacin (vitamin B3), and ascorbic acid (vitamin C) are involved in redox reactions. Thiamine (vitamin B1) and biotin are involved in the metabolism of micronutrients such as glucose and fatty acids. Moreover, cobalamin (vitamin B12), pyridoxine (vitamin B6), riboflavin (vitamin B2), and folic acid are involved in the synthesis of DNA, repair and regulation of cell division [35]. On the other hand, the fat – soluble vitamins such as vitamin D plays an important role in the innate immune system, through the production of antimicrobial peptides, including cathelicidin and defensins. This process is highly dependent on the status of tissues, which stimulate antibacterial activity in a variety of cell types by increasing the production of antimicrobial factors and by enhancing mechanisms associated with autophagy [27]. Vitamin K activates coagulation factors. Its severe deficiency causes bleeding [28]. The presence of both fat soluble vitamins A, E, D, and K and water soluble vitamins C and B groups in varying appreciable amount in the leaves of *A. garckeana*, is an indication of its richness in essential elements with potent antioxidants, antimicrobial, and antiviral activities. And since humans cannot synthesize B vitamins and need to introduce these nutrients with food [28], their presence in the leaves of *A. garckeana* is very essential for the promotion of health and wellbeing.

Minerals Compositions of *A. garckeana* Leaves

An adequate nutrition ensures a physiological growth while preventing diseases. Thus, micronutrients are essential: the lack of even one of them can have important consequences, impairing growth, delaying maturation or determining deficiency diseases such as rickets, scorbutus, and cretinism [28]. The highest

mineral concentration found in the evaluated leaves of *A. garckeana* plant was sodium (Na) 815.287±0.004 mg/100g, followed by calcium (Ca) 731.515±0.003 mg/100g, while the least concentration was copper (Cu) 1.048±0.008 mg/100g as shown in **Table 4**. Comparing this result with the one reported by Nkafamiya *et al.* [11] for *A. garckeana* fruits, leaves, roots, stem and bark. The contents of calcium and magnesium were correspondingly higher, while phosphorus contents are lower than all the results obtained for *A. garckeana* parts. Studies have shown that deficiencies in bioavailable Fe, Zn and other essential cation's minerals in human food, cause mineral malnutrition and affect a large proportion of the world population. Fe and Zn deficiencies are among the most prevalent micronutrient deficiencies in humans, affecting two billion people and causing more than 0.8 million deaths annually [36]. Iron (Fe) is an important component of hemoglobin and a vital element for the overall development of the human body. Calcium is essential for bones while zinc (Zn) helps in the overall development of the brain [24]. Copper is an essential micro-nutrient essential in the formation of bones, myelin sheaths in the nervous systems and helps in the addition of iron to the hemoglobin. It also assists in the transfer of iron from tissues to the plasma as well as its absorption in the gastrointestinal tract [37]. Potassium ions are very important in intracellular fluids and play a key role in acid-base balance, osmotic pressure regulation, and transfer of nerve impulse, contraction of cardiac muscles, cell membrane function and in glycogenesis. It also helps in the transfer of phosphate from ATP to pyruvic acid [37]. In general, vitamin D, and Zn regulate membrane fluidity, membrane integrity, gap-junction communication, and membrane repair. Vitamin D, and the trace elements Zn, Fe, Cu, and Se regulate the membrane-bounded antimicrobial peptide activities and mucosal-associated microbiota. The mucosal migration and regulation of immune cell functions also synchronize with the integrated pathways of vitamins B6, B12, and folate [38]. During early childhood, minerals and vitamins are essential for growth. After weaning, a varied diet is essential to obtain adequate micronutrient intake. Calcium, phosphorus and vitamin D are essential for bone growth; iodine allows thyroid hormones synthesis and brain myelination; iron is mainly necessary for the synthesis of red blood cells and new tissues. Finally, zinc is essential for the growth and regulation of the immune system [28].

Table 4: Concentration of Minerals in *A. garckeana* Leaves

Minerals	Values (mg/100g)
Calcium (Ca)	731.515±0.003
Magnesium (Mg)	531.665±0.012
Copper (Cu)	1.048±0.008
Iron (Fe)	10.958±0.007
Zinc (Zn)	7.274±0.003
Sodium (Na)	815.287±0.004
Potassium (K)	702.729±0.578
Phosphorus (P)	2.442±0.003

Results are expressed as Mean $\pm$ SD of three determinations (n = 3).

The Anti-nutrient Compositions of *A. garckeana* Leaves

Anti-nutritional factors are compounds which reduce the nutrient utilization and/or food intake of plants or plant products used as human foods. They play a vital role in determining the use of plants for humans [39]. Few anti-nutrients were found in the evaluated leaves of *A. garckeana* including considerable concentration of phytate 1.567 ± 0.005 mg/100g, and a least concentration of trypsin inhibitor 0.383 ± 0.006 Tiu/mg compared to oxalate 0.867 ± 0.003 mg/100g, hemagglutinin 0.741 ± 0.004 Hui/mg, and saponins 0.546 ± 0.004 mg/100g which were found in different concentrations showed in **Table 5**. The concentration of phytate 1.567 ± 0.005 mg/100g, is in accordance with the values of phytate in the leaf and root of *R. crispus* which were found to be $1.15 \pm 0.74\%$ and $1.38 \pm 0.27\%$ respectively [11]. On the other hand, the concentration of oxalate 0.867 ± 0.003 mg/100g in the evaluated leaf of *A. garckeana* was comparably higher than that of the leaf and root of *R. crispus* which were evaluated to have a deposit of calcium oxalate 0.15 ± 0.019 mg/g and 0.11 ± 0.0033 mg/g respectively [12]. Thus, the presence of these antinutrients in the evaluated leaves samples of *A. garckeana* plant may interfere with their bioavailability and decrease their nutritive values. For example, the presence of oxalate in food is known to interfere with the assimilation of nutrients, decrease the nutritive value of the food at high doses and may have adverse effects on human health [30]. Higher content of oxalate can bind to calcium present in food, thereby rendering calcium unavailable for normal physiological, and biochemical role such as the maintenance of strong bone, teeth, cofactor in enzymatic reaction, nerve impulse transmission, and as clotting factor in the blood. Furthermore, oxalates affects calcium and magnesium metabolism and react with proteins to form complexes which have an inhibitory effect in peptic digestion. Oxalic acid binds calcium and forms calcium oxalate which is insoluble. Calcium oxalate adversely affects the Ca absorption. Fatal human poisoning following the eating of large quantity of the leaves of certain plants i.e. rhubarb, known to contain relatively large amounts of oxalates has been reported [39]. Phytate (also known as Inositol hexakisphosphate (InsP6)) is the salt form of phytic acid and the primary storage form of phosphate and inositol in plant seeds. It forms complexes with dietary

minerals, especially iron, and zinc, and causes mineral-related deficiency in humans. It is of major concern for individuals who depend mainly on plant derivative foods. Phytate is of therapeutic use against diabetes mellitus, atherosclerosis, and coronary heart disease and reduces kidney stone formation, HIV-1, and heavy metal toxicity. However, information on the dosage for humans for eliciting beneficial effects is limited [39, 30]. Iron bound with phytate is presumably unavailable for absorption and uptake by the human intestine [40]. Sapogenin has hypo-cholesterolemic, anti-carcinogenic, anti-inflammatory, antimicrobial, antioxidant, antihaemolytic, and antibacterial activities. Sapogenins have been shown to have an inverse relationship with the incidence of renal stones. It binds these cholesterol-like compounds and thus interferes with their growth and division [30].

Table 5: Anti-nutrient Contents of *A. garckeana* Leaves

Antinutritional Contents	Values (mg/100g)
Phytate	1.567±0.005
Oxalate	0.867±0.003
Hemagglutinin (Hui/mg)	0.741±0.004
Trypsin inhibitor (Tiu/mg)	0.383±0.006
Saponins	0.546±0.004

Results are expressed as Mean ± SD of three determinations (n = 3).

In Vitro* Antioxidant Activities of Methanolic Leaves Extract of *A. garckeana

Assessment of antioxidant capacity of natural products has been regarded as a basis for ranking the antioxidant of plants and recommending best antioxidant foods for consumption. The evaluation of antioxidant activity of food and medicinal plants can be performed using chemical-based assays and cellular-based assays [41]. Antioxidants derived from food and medicinal plants have been increasingly investigated for their various nutritional function and health benefits [41]. There is therefore a growing interest in the protective role of medicinal plants as a better alternative to conventional antioxidants against free radicals and oxidative stress-associated diseases [42]. In this present research, the antioxidant potential of different concentrations of methanolic leaves extracts of *A. garckeana* plant were evaluated using ferric reducing antioxidants power (FRAP), and total antioxidants capacity (TAC). The methanolic leaves extract of *A. garckeana* exhibited higher ferric reducing antioxidant power (FRAP) activity across all the concentrations when compared to the standard antioxidant – butylated hydroxyl toluene (BHT). It has revealed that ferric reducing antioxidant power (FRAP) is concentration dependent with slight variations as shown in (Figure 2). There was non – significant different ($P>0.05$) in the concentration of the leaves extract compared to that of the standard antioxidant – butylatedhydroxyl toluene (BHT). The total antioxidants capacity (TAC) activities of the plant extract increased in

concentration when compared to the standard (ascorbic acid), and the highest activities was at 0.5µg/ml of extract (1.545 ± 0.523 µg/ml) considerably higher than that of the reference or standard antioxidant (ascorbic acid) which has the highest activity at 0.5µg/ml of extract (1.429 ± 0.497 µg/ml) as shown in **Table 6 & Figure 3** respectively. The inhibitory concentration (IC_{50}) of the crude extract of *A. garckeana* leaves was found to be (250.018 µg/ml), comparably higher than that of the reference or standard antioxidant - ascorbic acid (202.982 µg/ml) as shown in **Figure 3**. All the concentrations of the crude extracts of the leaves of *A. garckeana* were appreciably higher than that of the standard antioxidants (butylated hydroxyl toluene - BHT and ascorbic acid) in FRAP and TAC respectively. Which implies that the antioxidants activities of *A. garckeana* leaves extract was more potent at higher concentrations in both FRAP and TAC. The antioxidant potential of medicinal plants has been attributed to the redox effect of phenolic compounds which scavenge single oxygen, donate proton and act as reducing agents [24, 43]. Moreover, most of antioxidants usually have a single antioxidant property, like metal chelating ability or radical scavenging activity, which in many cases makes them inefficient to prevent oxidative flavour deterioration in foods ([44], and damage-related diseases by scavenging free radicals [45]. Hence, combinations of antioxidants exhibiting different antioxidant properties are commonly employed couple with an emerging interest to find natural plant-based antioxidants with multiple antioxidant properties [44]. In general, methanolic leaves extract of *A. garckeana* had high antioxidant activities as revealed in FRAP and TAC, which may be attributed to the high content of phenolic compounds and presence of aromatic compounds in *A. garckeana* plant. Thereby validating its potency as a natural plant based antioxidants with multiple antioxidants properties. Thus, providing scientific validation for their uses in the treatments of various human diseases in traditional medicine [32, 46, 47].

Conclusion

This study has shown that the leaves of *A. garckeana* have excellence nutritive values and antioxidants. They have been found to be high in carbohydrate and minerals with the exception of zinc, phosphorus, and copper; moderate in protein, fat and vitamins; and low in moisture contents; with potent antioxidants. The study further revealed low level of anti-nutrients such as trypsin inhibitor, saponins, oxalate and haemagglutinin, with moderate level of phytate. This has shown that the consumption of the leaves of *A. garckeana* plant can be of potent medicinal and dietary values. Therefore, utilization of the leaves of these underutilized wild edible plant may not only fulfil the nutritional needs, but will have a great role in nutraceutical development. However, more research is needed to provide adequate nutrient databases to promote its synergistic utilization in food and health.

Abbreviations

AG: *Azanza garckeana*

AOAC: Association of Official Analytical Chemists

AAS: Atomic Absorption Spectrophotometer

BHT: Butylated hydroxytoluene

DPIP: 2, 6-dichlorophenolindophenol

FRAP: Ferric ion Reducing Antioxidant Power

InterCEDD: International Centre for Ethnomedicine and Drug Development

SD: Standard deviation

TAC: Total antioxidants capacity

Declarations

Authors Statement/Contributions

Conceptualization: Chinelo Chinenye Nkwocha, Joshua Ogah Felix; resources: Joshua Ogah Felix; writing – original draft preparation: Chinelo Chinenye Nkwocha, Joshua Ogah Felix; review and editing: Chinelo Chinenye Nkwocha, Joshua Ogah Felix, Michael Lomi Oruchukwu; statistical analyses were done by Joshua Ogah Felix; supervision: Chinelo Chinenye Nkwocha and Joshua Ogah Felix. All authors have read and agreed to the published version of the manuscript.

Declaration of Conflict of Interests

The author(s) declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Plate

Plate 1 is available in the Supplementary Files section

Table

Table 6 is not available with this version

Figures

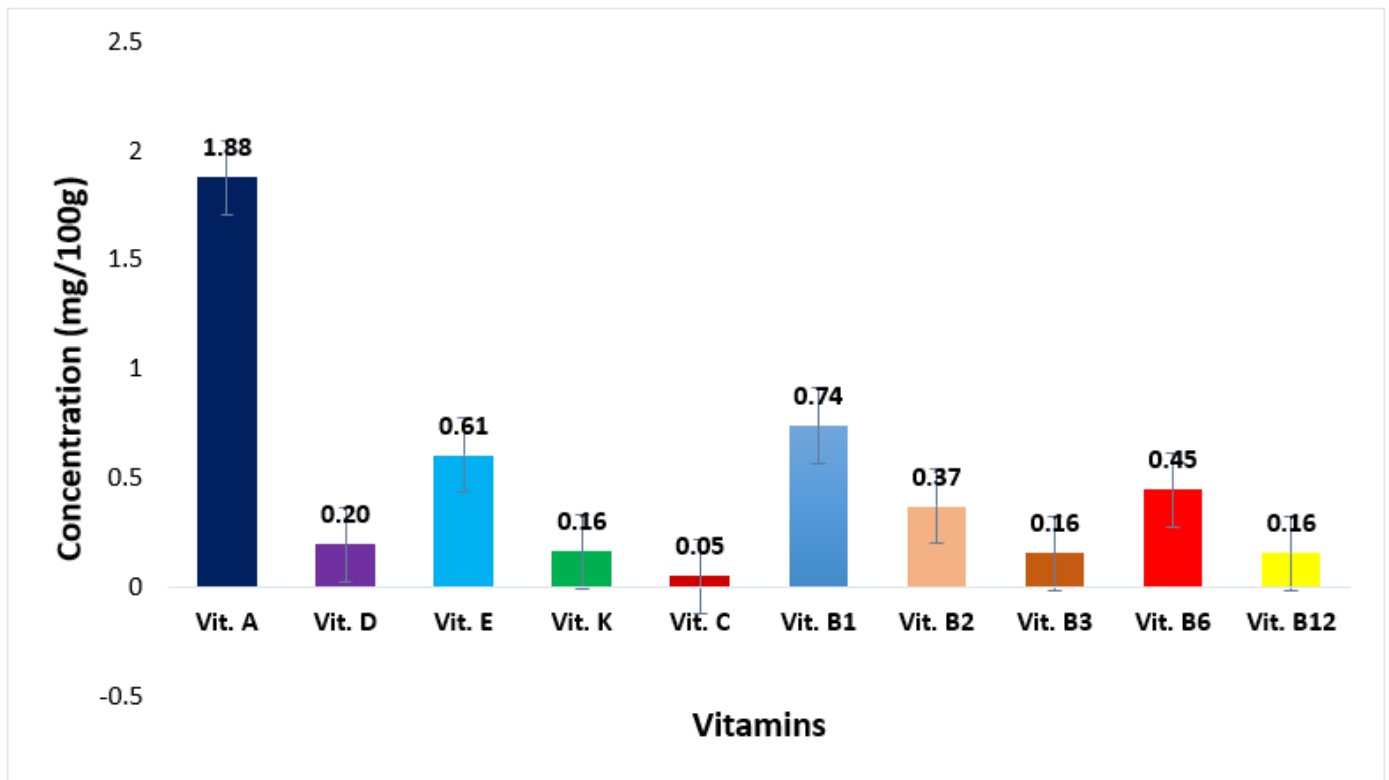


Figure 1

Compositions of fat soluble vitamins and water soluble vitamins of *A. garckeana* leaves

Values are presented as Mean $\pm$ SD of three determinants (n = 3).

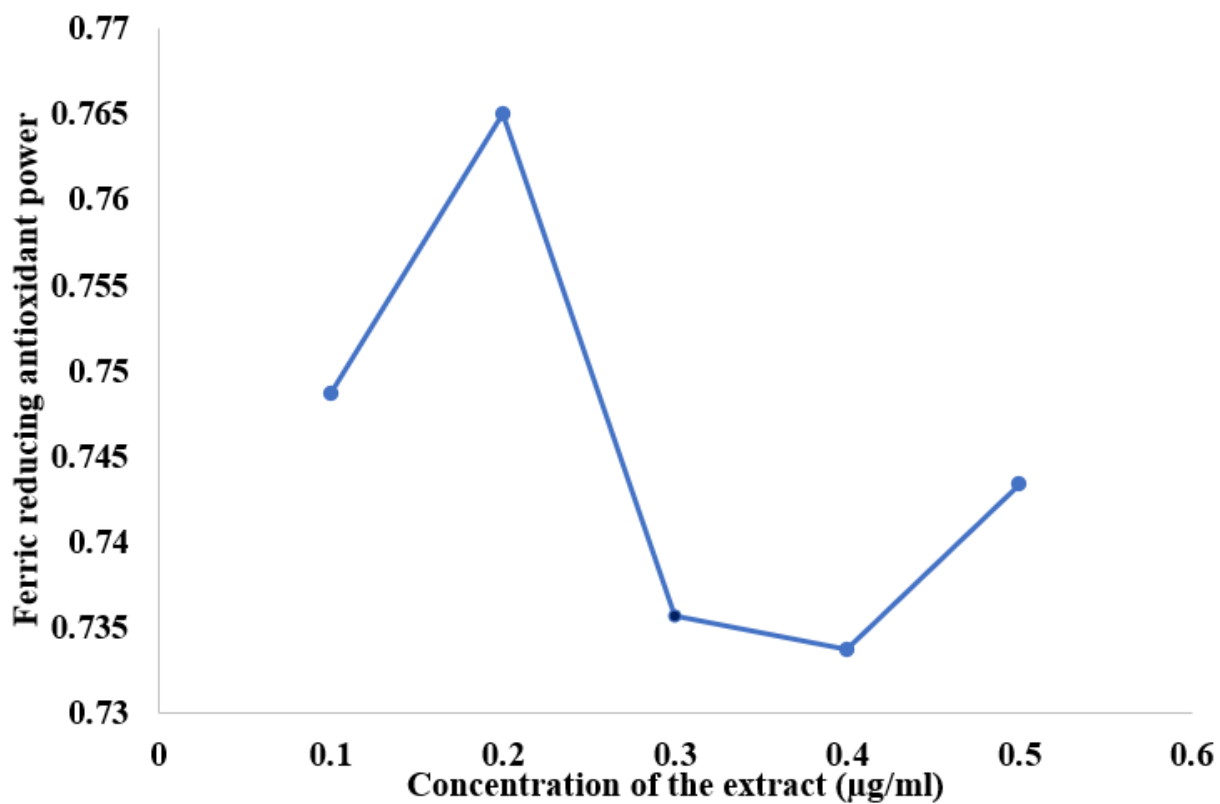


Figure 2

Ferric reducing antioxidant power of methanolic extract of *A. garckeana* leaves (% Inhibition against concentration of the extract).

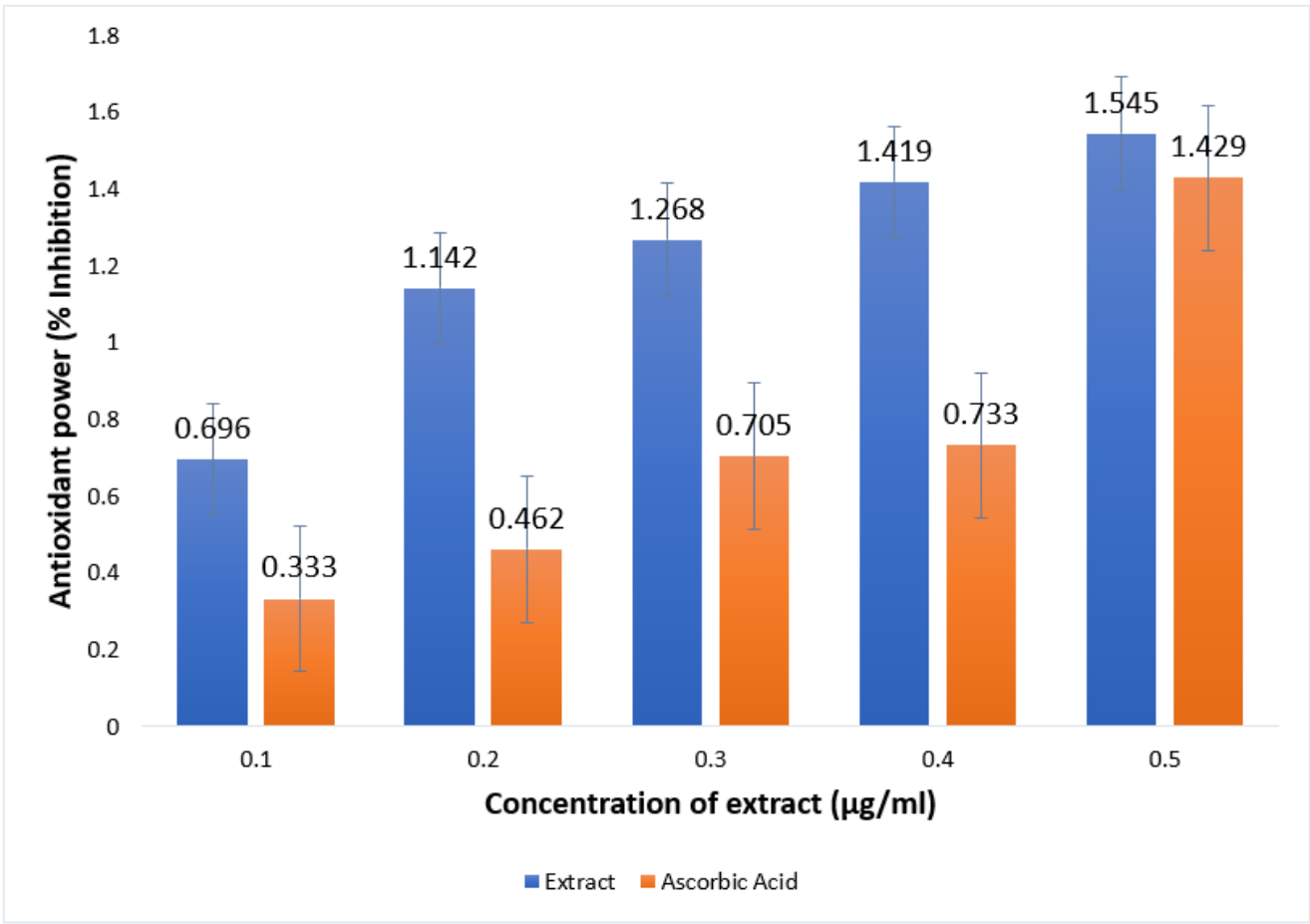


Figure 3

Total antioxidant capacity of methanolic extract of *A. garckeana* leaves (% Inhibition against concentration µg/ml).

Supplementary Files

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